THESES OF DOCTORAL (PHD) DISSERTATION

THE ROLE OF RENAL REPLACEMENT MODALITIES AND PHARMACOTHERAPY IN ATRIAL ARRHYTHMOGENESIS AND AUTONOMIC DYSREGULATION

by

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Members of the Examination Committee: István Kiss, MD, PhD, DSc
Balázs Ádám, MD, PhD

The Examination takes place at the 2nd floor Conference Room in the Bldg. of the Faculty of Public Health, University of Debrecen
11 a.m., 12 December, 2018.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
1 p.m., 12 December, 2018.

The epidemiology of atrial and ventricular arrhythmias

The frequency of cardiac arrhythmias has shown a growing tendency worldwide. Thanks to the constantly widening range of diagnostic instruments and the increasingly efficient therapy, life expectancy has become longer, involving a higher incidence of structural heart diseases. These tendencies facilitate the development of both atrial and ventricular arrhythmias, involving a growing frequency of these rhythm disturbances.

One of the cardiac arrhythmias with the highest clinical significance and growing incidence and prevalence worldwide is atrial fibrillation, affecting 0.5-1 % of the adult population. Atrial fibrillation is responsible for about one third of the hospitalizations due to arrhythmias; it significantly increases morbidity, mortality and health care expenditure.

The epidemiological studies spanning a period of several decades have confirmed that the frequency of atrial fibrillation exponentially grows with age. Its prevalence is 0.12-0.16 % among persons under 49; 3.7-4.2 % among those aged 60-70 and 10-17% among persons above 80 years of age. Up to the age of 75 its frequency is higher among men, but, since women have a higher life expectancy, 60% of the people above 75 suffering from atrial fibrillation are women, and the ratio of women and men is 1.2:1.

Ventricular arrhythmias also play an important role in the deterioration of mortality statistics. Sudden cardiac death (SCD), caused by malignant ventricular arrhythmias, is responsible for approximately 15-20% of deaths. In the United States its incidence is 180-450 thousand; its prevalence grows with age and its frequency is 2-3 times higher in the case of men than in women. Its incidence among men of fifty years of age is 100/100.000; among 75-year-old men it is 800/100.000. In Hungary, its prevalence is 1-2/1.000 patients, thus there are about 25.000 cases of sudden cardiac deaths a year. In about 80 % of the cases, ischemic heart disease can be identified as the underlying cause, while end stage kidney disease is also an important pathogenic factor, since the occurrence of SCD among hemodialyzed is 1.4-25%.

The pathogenic factors and electrophysiological background of atrial fibrillation

There may be several factors underlying atrial fibrillation, which often jointly trigger the electrophysiological processes playing a key role in arrhythmogenesis.

The uncoordinated atrial activation characterizing atrial fibrillation (atrial frequency: 400-800/minute) is often triggered by isolated or coupled atrial premature beats. The initial, so-called 'trigger' beat often occurs during bradycardia or after a longer pause. In the majority of the cases there is some anatomical and/or functional myocardial damage allowing the formation of a unidirectional impulse conduction block, which facilitates the development of a multiplex microreentry mechanism considered to be the electrophysiological substrate for atrial

fibrillation. This process, as a self-inducing cascade, may cause the repeated occurrence or permanence of arrhythmia.

Permanent atrial fibrillation induces several tissue alterations in the cardiac muscle referred to as structural remodelling. As part of this, substances stimulating collagen formation are released from the atrial myocardial cells. The emerging myocardial fibrosis, worsening with time, causes inhomogeneity in impulse conduction, triggers a change in the myocardial electrical activity and causes the regional inhomogeneity of depolarization and repolarization referred to as anisotropy, which may play a role in triggering another atrial fibrillation episode (anisotropic reentry). In the course of the secondary electrical remodelling triggered as a consequence of the structural changes, the activity of the L-type Ca^{2+}- and the outward transient K^+- ion channels decreases in the cardiac muscle cells, which shortens the length of the atrial action potential, atrial repolarization. The atrial effective refractory period shortens in parallel with the duration of the arrhythmia, enabling the development of another reentry circle, i.e. a "circulus vitiosus".

It has been proven that in atrial fibrillation the intra- and interatrial conduction time of the sinus impulse grows; furthermore, in the case of atrial impulse conduction disorder, the *P-wave duration* measured by 12-lead surface ECG is prolonged, and in such cases alterations can also be observed on the atrial signal-averaged ECG. Further examinations also confirmed that *P-wave dispersion* – the electrocardiographic marker indicating the inhomogeneity of atrial depolarisation – is prolonged in the case of patients suffering from paroxysmal atrial fibrillation but being in sinus rhythm, compared to the control group.

The unidirectional impulse conduction block occurring as a consequence of atrial structural and electrophysiological heterogeneity has a role not only in the generation of the re-entry but also in the development of the atrial premature beats, that also play an important role in triggering atrial fibrillation.

The inhomogeneous conduction of the sinus impulse, the development of atrial anisotropy may be triggered by several factors. These include the altered tissue structure of the atrial muscle, the pathogenic transformation of the action potential of muscle fibres, the increased vulnerability of the atrial myocardium, the enlarged ventricle and/or thickened ventricular wall, as well as the increased atrial volume and pressure load. The findings of previous studies reveal that various intra- and intercellular factors (e.g. angiotensin-II, TNF-α) can trigger conduction disorders, and *arrhythmogeneity may also be influenced by the altered functioning of the sympathetic and parasympathetic nervous systems*.

Complications of atrial fibrillation

Although the atrioventricular node transfers only a part of the supraventricular impulses to the ventricles, ventricular frequency may still be rapid (cca. 120-160/minute) and irregular, which may cause a sudden or slow deterioration in the hemodynamic status. The permanently shortened diastolic filling time (the stroke volume may be reduced by up to 15-25 %), the ventricular blood volume fluctuating from beat to beat may result in the dilation of the cardiac chambers, leading to systolic and diastolic heart failure and ultimately to the development of tachycardiomyopathy. This pathogenic process is greatly facilitated and aggravated by the lack of effective atrial contractions and the consequential occurrence of atrioventricular dissociation. In the dilated (left atrial transversal diameter> 40 mm), fibrillating atria functioning at extremely high frequency, blood flow slows down (known as atrial stasis), causing inclination to intraatrial clot formation (predilection location in the left atrial appendage). The broken off thrombus may cause embolization in the systemic circulation. It is to be noted that 70% of the cases of embolization of cardiac origin are cerebral embolism. The risk of a repeated cerebral event in the case of valvular atrial fibrillation is 17.6-times, in the case of non-valvular atrial fibrillation 5.6-times, while after stroke it is 12-times higher. There is also an increased risk of the development of stroke in the case of alternating sinus rhythm and atrial fibrillation. Considering these it is not surprising that the overall mortality of patients with atrial fibrillation is approximately 1.7-2.6-times higher compared to the population with sinus rhythm.

Cardiovascular events in the case of chronic kidney disease

Approximately 1% of patients treated with chronic kidney disease suffers from end-stage renal failure (glomerular filtration rate, GFR:<15ml/minute/1.73m^2), which involves the renal replacement therapy of some 2.5 million patients worldwide. As is well known, various cardiovascular complications (e.g. ischemic heart disease, arrhythmias, systolic and diastolic heart failure, stroke) play a significant role in the frequent and early death of people with chronic kidney disease. Compared to people with moderate kidney disease (GFR: 60-89 ml/minute/1.73 m^2), the occurrence frequency of cardiovascular events in stage three kidney disease patients (GFR: 30-44 ml/minute/1.73 m^2) is 2 times, in stage four kidney disease patients (GFR: 15-29 ml/minute/1.73 m^2) it is 3 times higher. 42.5% of the total mortality of patients administered hemodialysis can be attributed to a cardiac origin. People with chronic kidney disease face increased risk for the development of both atrial and ventricular cardiac arrhythmia; in the case of end-stage kidney failure 67.3 % of cardiac mortality, in the case of hemodialyzed patients 52 % can be related to malignant arrhythmias.

Atrial fibrillation and kidney disease

People with chronic kidney disease face an increased risk of the development of atrial arrhythmia, and thus atrial fibrillation. In a study published in 2010, the data of altogether 3,267 adult patients suffering from kidney failure but not yet administered dialysis treatment were analysed. The average GFR value of the participants of the study was 43.6 ± 13.0 ml/minute/1.73 m^2; in 55 % of those examined GFR was under 45 ml/minute/1.73 m^2. Atrial fibrillation was detected in 18% of the patients.

In the case of chronic kidney patients, there may be several causes underlying this atrial arrhythmia. The accumulation of what are known as uremic toxins, the various ionic and metabolic abnormalities (hyperkalaemia, hyperphosphatemia, hypocalcaemia, acidosis) play a significant role in triggering this arrhythmia.

As pathogenic factors, the *function disorder of the vegetative nervous system* (sympathetic overweight), *tissue hypersensitivity to catecholamines*, the altered activity of the baroreceptors, the accumulation of interstitial collagen, as well as ischemic heart disease (IHD) showing higher frequency can be identified.

The increased atrial wall tension caused by permanent or intermittent volume strain is also an important factor, as is also proven by the heightened level of brain natriuretic peptide (BNP) measured in kidney patients.

The increased activity of the renin-angiotensin-aldosterone system (RAAS), on the other hand, results in atrial myocardial fibrosis, which may have the permanence of the reentry mechanism as a consequence.

The pathogenic role of anaemia, malnutrition, secondary hyperparathyreosis and amyloidosis frequently occurring as consequences of kidney disease cannot be ignored, either. Endothelial dysfunction, increased oxidative stress or persistent inflammatory activity can also be assumed to be underlying arrhythmogenesis.

As an effect of all the above factors, the electric activity of atrial cardiac muscle cells may be altered, *depolarization-repolarization inhomogeneity* may develop, which may serve as the electrophysiological background for atrial arrhythmias.

Earlier examinations proved that hemodialysis could influence atrial electrical activity in itself, without further pathogenic factors, and thus induce atrial fibrillation. This can be put down to the fast ionic correction, the sudden termination of metabolic acidosis and the rapid decrease of wall tension observable in the course of the examination. It was also shown that, as regards atrial arrhythmia, the beginning of the kidney replacement treatment and the few hours thereafter constitute the critical period. In the case of kidney patients, it is primarily

thromboembolic complications that are responsible for mortality related to atrial fibrillation; at the same time, the role of adverse hemodynamic consequences should not be neglected, either.

Hemodialysis and hemodiafiltration

In order to improve the treatment of end-stage kidney patients, a novel renal replacement therapy called hemodiafiltration was introduced. The special significance of this modality is that the mortality rate of patients receiving hemodiafiltration is 35 % lower than that of patients participating in traditional hemodialysis programmes.

Contrary to conventional hemodialysis – which eliminates uremic toxins primarily depending on their molecular weight, by diffusion – hemodiafiltration serves, by way of what is referred to as *convective transport,* the elimination of toxic polypeptides of medium molecular weight (characterised by β_2-microglobulin) as well.

During hemodiafiltration so-called high-flux filters with high ultrafiltration coefficient are used, as a result of which ultrafiltrate of a great volume is produced. Considering this the substitution of the ultra-clean dialysate produced by the dialyser is required. In the clinical practice, the method known as post-dilution is used, in the course of which the substitution solution is led back, after the filter, to the venous system. In the course of a treatment taking 4 hours on the average, dialysing solution of approximately 18-24 litres has to be substituted.

There have been several studies examining the safety and efficiency of hemodiafiltration. It has been confirmed that despite the high ultrafiltration rate observed during hemodiafiltration, the number of intra- and inter-diafiltration hypotensive periods falls. Patients tolerate the treatment more and their quality of life improves. The background of this phenomenon has not been clarified precisely yet; the role of the more efficient removal of the vasodilating agents and, through that, the favourable modulation of peripheral vasomotor activity arises. The high sodium content of the substitution fluid may also be a triggering factor in the prevention of hypotension. Thanks to the above mechanisms, fatigue following treatment eases, which has clinical advantages primarily in the case of patients with high cardiovascular risk.

80-100% of the patients receiving traditional hemodialysis develop anaemia requiring the administration of eritropoetin. In the case of hemodiafiltration, anaemia becomes less frequent, so the patients treated require less eritropoetin. There are still questions about the exact mechanism of this phenomenon; it is assumed as a possibility that in the course of the application of this treatment, the factors preventing blood formation are removed from the body.

The reduction of inflammatory complications may also contribute to the favourable change in the frequency of anaemia since the concentration of acute phase proteins, inflammatory mediators (C-reactive protein, interleukin-1, interleukin-6, rheumatic factor) does

not rise either during or after the treatment. It must be emphasised that during hemodiafiltration oxidative stress falls and the lipid profile improves.

The decrease in the β_2-microglobuline concentration reduces the incidence of amyloidosis by approximately 50 %.

While in the course of conventional renal replacement therapy peripheral uremic neuropathy may infrequently develop, the easing of neurological symptoms is reported in the course of convective treatment.

In view of these favourable effects and the improvement of the mortality rate, hemodiafiltration is considered to be the most efficient and most modern renal replacement therapeutic procedures these days.

The autonomic innervation of the heart and its role in atrial arrhythmogenesis

The heart has its own independent impulse generation and conduction system; its vegetative innervation is expressly rich. There are both sympathetic and parasympathetic fibres running towards the atria, while ventricular innervation is decisively sympathetic.

The fibres of the sympathetic nervous system originate from the posterior and lateral nuclei of the hypothalamus and end in the paraspinal ganglions. From here the fibres run, from the spinal cord segments Th1-5 towards the cardiac plexus. The axons of the nerve cells synapse in the stellate ganglion and along the great arteries and coronaries they reach the cardiac muscle. In the case of sympathetic nervous system excitement noradrenalin as a neurotransmitter is released from the nerve ends, which affecting the cardiac muscles, primarily the β_1-receptors, increases intracellular Ca^{2+}-concentration, as a consequence of which the contractility of cardiac muscle cells increases (inotropy). In addition, in the case of enhanced sympathetic tone, heart frequency (chronotropy) rises, conduction speed (dromotropy) grows, the irritability of the ventricular working muscles (batmotropy) increases and muscle relaxation (lusitropy), too, becomes faster.

The parasympathetic fibres originate from the nuclei of the medulla (the nucleus ambiguus, the nucleus of the tractus solitaries and dorsalis motoneurons) and pass along the vagus nerve. The parasympathetic neurons reach the epicardial fat pad at the borderline of the right pulmonary vein and the left atrium, at the ligation of the inferior vena cava to the right atrium, and near the superior vena cava. From here, the fibres run towards the sinus node and the atrioventricular node. While the right vagus nerve innervates rather the sinus node, the left one innervates the atrioventricular node to a greater extent. The acetylcholine released from the cholinergic fibres exercises its impact on the myocardial muscarinic (M_2) receptors. Vagal excitement triggers the release of K^+ from the cells, which results in the hyperpolarization of the cell membrane. Under physiological circumstances, the vagal tone is constant, which slows

down the spontaneous impulse generation from the resting sinus node to some extent. This is well illustrated by the fact that after the administration of parasympatholytic medication like e.g. atropine, heart frequency rises.

In addition to the above, what are known as mechano- and chemosensitive receptors are also present in the myocardium and epicardium, which transfer afferent impulses to the central nervous system.

As the findings of earlier studies reveal, the vegetative nervous system also plays an important role in triggering and maintaining atrial arrhythmias (e.g. sinus tachycardia, atrial tachycardia, atrial fibrillation). It has been observed that in the paroxysmal or lone atrial fibrillation of young patients (provided there are no cardiac risk factors and/or structural heart diseases in the background), there is increased vagal tone. In the case of structural heart disease, on the other hand, sympathetic nervous system activation is dominant. In the case of post-operative patients it has been established, by analysing heart rate variability data obtained by Holter ECG, that the activation of the sympathetic nervous system and the reduction of the parasympathetic tone play a role in the development of both paroxysmal atrial fibrillation and atrial flutter characteristic in the post-operative period.

The role of gamma-aminobutyric acid (GABA) in the central nervous system

Gamma-aminobutyric acid is an amino acid found both in the central nervous system (CNS) and the spinal cord. In the central nervous system it is present primarily in local short interneurons, while in the spinal cord it participates in pre-synaptic preventive processes. GABA is one of the most important inhibitory neurotransmitters in the central nervous system.

GABA is synthesized from glutamate, as an effect of the glutamate-decarboxylase enzyme. It is released from the synaptic vesicles through calcium-dependent exocytosis. The suspension of its effect can be put down to both the diffusion from the synapse and the reuptake processes. It is broken down by the enzyme GABA-transaminase.

According to our current knowledge, there are three types of GABA-receptors. The receptors $GABA_A$ and $GABA_C$ are ionotropic receptors composed of several subunits. The $GABA_B$ receptor is a G-protein dependent metabotropic receptor composed of 7 transmembrane proteins. $GABA_A$ receptor is in fact a Cl^- channel that activates after the binding of GABA as agonist, after which the Cl^- ions, depending on their concentration gradient, flood into the cells, thus resulting in hyperpolarization. $GABA_A$ receptors have a pentamer structure (are composed of 5 subunits) and several allosteric binding sites, thanks to which they are able to bind not only GABA molecules but also e.g. barbiturates, benzodiazepines (BDZ's) and neurosteroids. The subunits come in several types (e.g. α, β, γ,), which theoretically results in great structural variation. Not all combinations are stable and some of them, e.g. the central

nervous system ((α1)2, (β2)2, γ2) are much more frequent than others. At various parts of the brain GABA$_A$ receptors of various structures are expressed. Unfortunately we are still only partially familiar with the subunit composition of native GABA$_A$ receptors. This variability may form a molecular basis for the somewhat different effect spectrums of the various chemicals.

According to a possible GABA$_A$ receptor model the binding site of GABA is on the β-subunit. This is also the binding site of the antagonist bicuculline (which prevents the binding of the GABA). On the α-subunit you can find the binding places of ethanol, barbiturates, anaesthetics and picrotoxin (the Cl$^-$channel blocker). The binding sites of benzodiazepines are present on the γ-subunit (ω-receptor). Benzodiazepines by themselves are unable to activate GABA$_A$ receptors even in relatively large concentration. The modulation effect is apparent in that they increase the frequency of the opening of the Cl$^-$-channel, i.e. the channel will open more frequently. There are BDZ receptor inverse agonist substances as well (β-carbolin, Ro 15-4513), which reduce influx from the Cl$^-$-channel, whereby they, as negative modulators, may cause anxiety and inclination to spasm. In higher concentration, barbiturates are able to activate GABA$_A$ receptors by themselves, at the same time; they prolong the opening time of the channel. GABA$_A$ receptors may play a role in the development of the effect of several other medication or narcotic drugs on the central nervous system (e.g. general anaesthetics like propofol, halothane).

GABA-erg neurons have a significant role in sensation, in triggering the appropriate sensomotoric response and in maintaining memory and the state of consciousness. They facilitate falling asleep and improve the quality of sleep as they have muscle relaxing and sedative effects as well. They mitigate anxiety and mood swings, and have a painkilling character as well. In addition to the above, the GABA-erg system influences (through the ambiguous nucleus and the vagal nerve) the functioning of the cardiovascular system, both directly and indirectly. It controls the vascular tone, thereby also regulating blood pressure and heart rate.

Effect of benzodiazepines

Benzodiazepines (BDZ) exercise their effect through the GABA$_A$ receptor (γ-subunit, ω-receptor); they prolong the opening frequency of the Cl$^-$-channel, thereby causing hyperpolarization, which inhibits the effect of transmitters stimulating neurons. The chemical substance competitively inhibiting the binding of BDZ is flumazenil.

BDZ-derivatives are lipid soluble agents; in the case of oral administration they practically get absorbed completely. The time between the agents are administered and maximum plasma concentration is reached ranges approximately between 30 minutes and 4

hours; the majority significantly associate to plasma proteins. The more lipophilic a BDZ-derivative is, the faster it gets into the brain tissue. Diazepam is strongly lipid soluble, so its effects and by-effects manifest themselves sooner. At the same time, its distribution in the peripheral tissues is faster and thereby its effect peters out sooner. Lorazepam is a less lipophilic substance and thus its effect develops more slowly but is longer lasting. BDZ's get into the foetus and breast milk as well.

The metabolism of benzodiazepines is characterised by both oxidation and conjugation processes. During oxidation, active metabolites may be created; the majority are metabolised this way (e.g. diazepam). During conjugation, certain derivatives are associated to glucuronic acid (e.g. lorazepam). The oxidation process may be influenced by age and liver function to a significant extent. In older age, elimination significantly decreases, while sensitivity usually grows; this may make it necessary to reduce the dose and rationing requires special carefulness in the case of long-effect agents. Benzodiazepines are emptied from the body through the urine.

Anxiolytic effect of benzodiazepines

The chemical substances in the family of benzodiazepines are all effective *anxiolytics*; in small doses it is mainly this characteristic feature that dominates. *Alprazolam* is considered to be the most selective anxiolytic. Based on the strength of the anxiolytic effect, benzodiazepines can be divided into two groups. "Low potential" benzodiazepines are older, classic agents whose effective dose is above 10 mg (e.g. diazepam, medazepam). These are not effective in paroxysmal states of anxiety. "High potential" agents are effective in a dose of under 10 mg as well (e.g. alprazolam, lorazepam, clonazepam) and are also suitable for the treatment of panic attacks. It is important to establish that even in the case of permanent treatment no tolerance develops to the anxiolytic effect of benzodiazepines.

Further clinical effects of benzodiazepines

Benzodiazepines have a *hypnotic* effect as well, changing the structure of physiological sleep. They shorten the time required for falling asleep and prolong the term of sleep. Sleeping pills (e.g. flunitrazepam) whose plasma concentration decreases more slowly often cause sleepiness, attention deficit or coordination problems after awakening in the morning.

Benzodiazepines may also cause an *anterograde amnesia*. Characteristically patients do not remember the events prior to falling asleep or after waking up.

They are effective *anticonvulsants*; furthermore, they also have a central *muscle relaxant* effect. It can be established at the same time that there fast develops tolerance against the anticonvulsant effect.

As regards the side effects it must be stressed that they may trigger tiredness, memory and *attention failure, the deterioration of learning skills.* Infrequently – as a paradox phenomenon – *increased activity, anxiousness* may occur.

The effect of benzodiazepines on the cardiovascular system should not be neglected, either, as in higher doses they may cause a *fall in blood pressure* and consequential *tachycardia and breathing depression.*

The pathomechanism of benzodiazepine withdrawal syndrome

There is evidence proving that the regular administration of benzodiazepines on a daily basis and in high doses causes addiction, somatic and psychological dependence in the long run. The risk of developing benzodiazepine dependence drastically rises by increasing the daily doses and the length of treatment, and is higher in the case of those whose medical history includes alcohol, drug and/or medication addiction.

Binding to the γ-subunit of the GABA$_A$ receptor, benzodiazepines increase the opening frequency of the Cl$^-$-channel, thereby enhancing hyperpolarization in the neurons, inhibiting their activity. Thanks to the above mechanism, in the case of the long-term administration of BZD, in certain parts of the central nervous system (e.g. substantia nigra) the density of the surface GABA$_A$ receptors decreases (down regulation). Beyond this, several other hypotheses, too, have emerged in the background of the changing nervous system response to the long-term administration of benzodiazepines. Among others, in certain central nervous system locations the conformational change of receptors, the modification of their metabolism and the decreased expression of receptor genes have been observed. Furthermore, the loss of contact between the GABA and BZD binding places and the decreasing GABA synthesis may also play a role in the neuro-adaptation, the tolerance developing in the long-term administration of BZD. At the same time, a compensatory rise in the production of glutamate as an important excitatory neurotransmitter takes place, which intensifies in the case of sudden BDZ withdrawal, whereby the sensitivity of the CNS grows.

The clinical consequences of benzodiazepine withdrawal

Long-term and high-dose BDZ treatment may cause dependence, whereby the sudden withdrawal of these agents may involve what is known as a *withdrawal syndrome*, which may manifest itself primarily in the clinical picture of *tension, tremor and sweating.* Sleeping becomes superficial and non-relaxing. *Muscular cramps, muscle twitch* and, infrequently, even *epileptic seizures* may occur. *Palpitation*, *panic attacks* involving shortness of breath can be

observed; what is more, the longing for BDZ's sometimes becomes unbearable. The patient may become both physically and psychologically exhausted. In the few months after the acute period of BDZ withdrawal (cca. 10-14 days) frequent *mood swings, fear, depression, excitement, panic attacks, attention deficit or sexual deficit* may be characteristic, while *cognitive dysfunctioning* and *forgetfulness* may also occur. As a consequence of the prolonged psychological-emotional instability many patients are unable to fight the withdrawal symptoms and start using BDZ derivatives, whereby the vicious circle closes.

Pathomechanism and clinical characteristics of pseudopheochromocytoma

Pseudopheochromocytoma is a syndrome triggered by the hyperactivity of the sympathetic nervous system and a rare reason of malignant hypertension. Its symptoms may include: sudden, paroxysmal rise of blood pressure, palpitation, chest pain, intense sweating, tremor, headache, vertigo and temporary mental disturbance. A seizure may last between several minutes to several hours.

Pheochromocytoma as a well-known endocrine medical condition may also cause secondary hypertension occurring in attacks. There is usually a tumor originating in the adrenal medulla in the background, 10% of which are malignant. The sudden catecholamine release from the tumor results, among others, in significant blood pressure rise. In 24-hour urine, catecholamine degradation products (metanephrine, normetanephrine, serotonin, 5-hydroxi-indol-acetic acid) can be detected, while during the respective attacks, higher catecholamine levels can be detected in the serum. These help setting and differentiating the diagnosis.

The reason underlying pseudopheochromocytoma is *not a catecholamine secreting tumor*, however, since between the respective episodes or during paroxysm catecholamine degradation products cannot be measured or can be detected just in minimum amounts in the blood or urine. Several clinical conditions may lead to pseudopheochromocytoma. It must be emphasised that the administration of certain pharmacological agents (e.g. sympathomimetics, tricyclic antidepressants) may involve pseudopheochromocytoma thanks to the activation of the sympathetic nervous system.

The research question

As an internal medicine specialist and PhD student at the Institute of Internal Medicine of the Clinical Centre of the University of Debrecen, my interest was drawn towards the pathology, diagnostics and treatment of cardiac arrhythmias as well as to opportunities to estimate the risk of arrhythmias. In the course of my activity as a doctor, I often meet end-stage kidney patients and studying these patients' medical history has been highly influential in guiding my scientific activity towards the border area of cardiology and nephrology. In commencing my research I was also stimulated by the fact that the role of hemodiafiltration in arrhythmogenesis had not been clarified yet and it was similarly unknown whether the lower frequency of arrhythmia was a reason underlying the more favourable mortality statistics.

In the course of my medical specialist activity in emergency and intensive therapy care I had the chance to participate in the examination and treatment of a patient whose acute deterioration of condition was caused by vascular tone regulation disorder occurring as a consequence of sympathetic nervous system activation, serious hypertension, cerebrovascular disorder and sinus tachycardia. Underlying this serious, life-threatening medical condition, the sudden termination of previous regularly administered anxiolytic therapy was confirmed. In the second part of my investigations, I studied the pathomechanism of this rare and serious medical condition requiring immediate care, referred to as *pseudopheochromocytoma*, and its correlation with pharmacotherapy.

Scientific objectives

In order to prevent arrhythmia in the case of patients with end-stage kidney failure, and with the objective to clarify the development mechanism of certain arrhythmias, I sought to find answers to the following questions in the specific issues.

Studying the electrocardiographic markers characterising atrial electrical activity during hemodialysis and hemodiafiltration

a) Are there any anomalies observable with reference to the change of the *P-wave duration* and *P-wave dispersion* during hemodialysis and hemodiafiltration?

b) Are there any differences between the two kidney replacement therapies with reference to atrial arrhythmias, based on the quantitative and qualitative arrhythmia analysis of the Holter-electrocardiogram data?

c) Is there a significant correlation between the respective laboratory markers (especially as regards the electrolyte values) and the atrial electrocardiographic parameters?

d) Examination of the correlation between the echocardiographic parameters and atrial electrocardiographic markers.

Examination of the effect of anxiolytic withdrawal on the cardiovascular system

In examining the serious clinical syndrome occurring as a consequence of anxiolytic withdrawal, known as *pseudopheochromocytoma*, I sought to find answers to the following questions.

a) Studying the effect of the sympathetic and parasympathetic autonomic nervous system tone on the cardiovascular system and on arrhythmogenesis.

b) How do benzodiazepines affect the cardiovascular system through the GABA-erg system?

c) What mechanism can characterize benzodiazepine-withdrawal and how can it be described from the clinical aspect?

d) Studying the role of *alprazolam-withdrawal* in triggering pseudopheochromocytoma in relation to one of our cases, with special respect to vascular tone regulation and atrial arrhythmogenesis. *Attempting to detect still unidentified pharmacological mechanisms underlying the clinical syndrome.*

Patients and methods

End-stage kidney patients

In our study we examined the data of 30 end-stage kidney failure patients requiring renal replacement therapy (men: 18, women: 12; average age: 60.57 ± 13.62 years: from 23 to 85). Underlying the chronic kidney failure, hypertensive and vascular nephropathy and chronic glomerulonephritis were detected in the majority of cases.

As the first step, we collected and evaluated the data of patients administered hemodiafiltration for at least 3 months. Thereafter we continued the kidney replacement treatment of the same patients with conventional hemodialysis for more than 3 months, after which we collected and analysed data once again. During selection for the study, clinical conditions affecting cardiac impulse generation and conduction (e.g. diabetes mellitus, hemochromatosis, sarcoidosis, amyloidosis, Parkinson-disease, carcinoid) constituted exclusion criteria. None of our patients had atrial fibrillation in their medical history. Pharmacological treatment influencing ECG-based arrhythmia markers (e.g. amiodaron, sotalol, macrolide antibiotics, antifungal agents, haloperidol, selective serotonin reuptake inhibitors) was also an excluding factor. Participation in the study was also excluded in the case of thyroid gland dysfunction (hyper- or hypothyroidism) or Ca^{2+} metabolic disorder.

27 of the patients suffered from hypertension (arterial blood pressure > 140/90 mmHg), dyslipidemia was detected in the case of 5 patients (serum cholesterol> 5.2 mmol/l), and three of them suffered from ischemic heart disease confirmed by stress test. One patient had earlier undergone surgery due to aorta stenosis. Having received and comprehended detailed information on the study, the patients confirmed their intention to participate in writing. The study protocol was approved by the Ethics Committee of the University of Debrecen.

Hemodialysis and hemodiafiltration

The renal replacement therapies were performed with Fresenius 4008-S and H devices (Fresenius Medical Care, Bad Homburg; Germany), Fx60 and Fx80 „high-flux" polysulfone dialysis capillaries (Fresenius). The treatments took 3x4 hours a week. During hemodiafiltration, the post-dilution method was used. The dialysis bicarbonate solution contained 138 mmol/l of sodium, 1.5 mmol/l of calcium, 0.5 mmol/l magnesium and 1 g/l glucose, as well as, in 13 cases 2 mmol/l, and in the other 17 cases 3 mmol/l potassium. The patients' previous pharmacological therapy (angiotensin-converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), β-receptor blocker, Ca^{2+}- antagonist, Digitalis, nitrate) was not modified. On the day of the examinations the patients were not administered any other drugs but sodium- heparin. In the course of the renal replacement treatments, arterial blood pressure was measured by a non-invasive method. The speed of blood flow was 338 ± 11.6 ml/minute,

and with reference to this there was no significant difference between the two modalities ($p < 0.05$).

12-lead electrocardiography and renal replacement therapy

In the course of our examinations we performed 12-lead, surface ECG on 5 occasions altogether: at the beginning of the renal replacement therapy, at minutes 15; 30; 240, and 2 hours after the end of the treatment. The simultaneous 12-lead electrocardiograms were registered with a Hewlett Packard Page Writer 200i type, 12-channel ECG device. We used 25 mm/sec sheet running speed; the patients were lying on their backs, they did not talk and were breathing calmly. To avoid inter-observer variability, in the calculations we used data calculated by an examiner who was unfamiliar with the origin of the record chart concerned. The record charts were amplified by a factor of three. In the case of each lead we measured three consecutive P-waves with a compass, calculated the average of the values and defined the result obtained as the P-interval of the lead concerned. In the statistical examinations, we marked the longest P-distance of the 12 leads as *Pmax*, and the shortest as *Pmin*. From the difference of the two we derived the *P- dispersion* (*Pd*). For the calculation of the P-interval corrected for the base frequency (*Pmaxc*) we used the Bazett's formula (*Pmaxc* = P / √RR (msec)), and the corrected P-dispersion value t (*Pdc*) was also calculated with the Bazett's formula (*Pdc* = *Pd* / √RR (msec)).

Examination of kidney patients using Holter-electrocardiography

For the qualitative and quantitative examination of atrial arrhythmias there was a Holter-ECG performed in the case of every patient (GE Medical SEER Light). The device was installed before starting the renal replacement therapy; the registration period lasted 24 altogether and thus we were able to examine also the frequency and types of arrhythmias occurring during the intra- and interdialytic periods as well. In addition to the absolute number of atrial premature beats we also established the premature beat to total ventricular beat ratio. Our purpose with the latter was to eliminate the differences arising from the short differences (of a few minutes) of the examinations.

Laboratory examinations during renal replacement therapy

In the course of the renal replacement therapies we measured the concentration of several ions four times (at minutes 0; 15; 30 and 240) and 2 hours after finishing treatment. We examined the sodium, potassium, total calcium, ionised calcium, phosphate and magnesium levels of the serum.

Echocardiographic examinations on kidney patients

In every case, before and after the renal replacement therapies we performed transthoracic echocardiography on 2 occasions (M-mode, 2 D). In the Doppler examinations we used pulsatile and continuous wave technique (by means of Philips ATL HDI 5000 imaging system and 3.5 MHz transducer). From the parasternal longitudinal view we specified the left atrial cross diameter, the thickness of the interventricular septum and the left ventricular posterior wall and, using the Devereux-Reichek's formula, calculated also the left ventricular mass index ({1,04 x [(left ventricular end diastolic diameter + thickness of the interventricular sept + thickness of the left ventricular posterior wall)^3- left ventricular end diastolic diameter^3]- 14} / body height). The ejection fraction (EF) representing the left ventricular systolic function was determined from the apical four-chamber section using the Simpson's method. When examining the left ventricular diastolic function we characterized the early diastolic mitral peak velocity (E) using pulsed wave Doppler technique, and measured in the course of atrial contraction the maximum value of the late diastolic flow velocity (A). We defined the duration between the peak and the end of the deceleration slope of the E-wave as deceleration time (DT). In the course of tissue Doppler imaging (TDI) we determined the septal longitudinal velocity of the mitral annulus (Ea) and on the basis of the E/Ea ratio we estimated left ventricular filling pressure as well.

Statistical analysis

The statistical analysis was performed using the SAS 8.2 for Windows programme. The change of the variables over time and the difference between the two renal replacement methods were examined by using repeated measurement ANOVA (Analysis of Variance). The correlation between the parameters was analysed by Pearson's test in the case of normal and Spearman's test in the case of abnormal distribution. In our examinations we considered the probability level of $p < 0.05$ as significant.

Findings obtained during the renal replacement therapies
P-wave and renal replacement modalities

In the course of hemodiafiltration, at the beginning of the treatment already, the *Pmax* and *Pmaxc* values were significantly higher than during conventional treatment (*Pmax*: 102 msec vs. 88.66 msec, $p = 0.0036$; *Pmaxc*: 114.83 msec vs. 99.4 msec, $p = 0.0036$). At the same time, all the initial values were within the physiological range. The atrial electrographic markers examined (*Pmax*, *Pd*, *Pmaxc* and *Pdc*) were significantly prolonged, compared to the starting

values, already in the 30th minute of the hemodialysis ($p < 0.05$). No significant change similar to this was experienced during hemodiafiltration.

The average *Pmax* value was 88.6 msec at the start of hemodialysis, rose to 102 msec ($p < 0.05$) by minute 30; by minute 240 of the treatment it reached the highest value (111 msec, $p < 0.05$), and finally, 2 hours after the treatment it dropped to 98 msec.

At the start of the hemodialysis, a *Pd* value of 37.3 was measured, which was prolonged to 52 msec ($p < 0.05$) in the first half an hour of the treatment already.

The *Pmaxc* value rose from the starting value of 99.4 msec to 115.5 msec ($p < 0.05$) and still had a heightened value 2 hours after the end of the hemodialysis (115.3 msec).

In the first half an hour of the hemodialysis, the *Pdc* did not change significantly (44 msec), but later it fell to 40.4 msec ($p < 0.05$), and by the end of the treatment its value rose (48 msec, $p < 0.05$). Two hours later the *Pdc* value dropped to the starting value (43.9 msec).

Contrary to the above, these P-wave parameters did not show any significant changes during hemodiafiltration.

Laboratory parameters of our kidney patients

The total calcium (tCa) and ionised calcium (iCa^{2+}) levels of the serum rose, while the potassium, magnesium and phosphate concentrations showed a decline in the case of both types of treatment. During hemodiafiltration the sodium level did not change significantly, while temporary declines were observed in minutes 15 and 30 of the hemodialysis ($p < 0.05$), after which the sodium level rose to the starting value by the end of the treatment.

We determined the glucose concentration level of the serum at the beginning and end of both renal replacement therapies and observed a significant rise (HD: 5.69 ± 1.16 mmol/l vs. 6.81 ± 1.53 mmol/l, $p = 0.0079$; HDF: 5.25 ± 0.72 mmol/l vs. 7.07 ± 1.58 mmol/l, $p < 0.0001$). At the same time, no significant correlation was observed with reference to the electrocardiographic parameters or serum glucose.

The value of Kt/V indicating the efficiency of kidney replacement treatments was 1.35 ± 0.17 in the course of hemodialysis and 1.39 ± 0.21 during hemodiafiltration. These parameters did not differ significantly in the case of the two types of treatment ($p = 0.3$).

Correlations between the atrial electrocardiographic markers and laboratory parameters

During hemodiafiltration, the change in the sodium concentration of the serum showed a significant positive correlation with the *Pd* and *Pdc* values. Remarkably, during traditional hemodialysis it was ionised calcium that positively correlated with the *Pd* and *Pdc* parameters.

At the beginning of the renal replacement therapies we also determined the bicarbonate values of the serum, but we observed no significant differences between the two modalities with reference to this parameter (HD: 19.6 ± 2.1 vs. HDF: 20.7 ± 2.8; $p = 0.14$).

In every case we adjusted the bicarbonate concentration of the dialysing solution to individual needs (within a range of 28-36 mmol/l) so as to make sure that the bicarbonate value of the plasma was between 20-22 mmol/l. Thereafter we did not make any further changes to the bicarbonate concentration of the dialysing solution.

We also examined if bicarbonate concentration before the treatment showed any significant correlation with the P-wave parameters. In this respect, a significant negative correlation was detected during hemodialysis between the end-of-treatment *Pmax*, *Pd* and *Pdc*, and the beginning-of-treatment bicarbonate values. Apart from the above we found no other significant correlation between the ions and the atrial electrocardiographic markers.

Changes in body mass and volume status during the various renal replacement modalities

Body weight and the body mass index (BMI) significantly fell in the course of both renal replacement modalities (average decrease in BMI HD: 24.39 ± 4.19 kg/m^2 vs. 23.59 ± 4.2 kg/m^2, $p < 0.05$; average decrease in BMI HDF: 24.37 ± 4.12 kg/m^2 vs. 23.6 ± 4.14 kg/m^2 , $p < 0.05$). We determined the change in body weight respectively according to gender, and established that in both cases there was a significant fall ($p < 0.05$). It must be emphasised that with reference to body weight and BMI change there was no significant difference between the two renal replacement methods. This also means that the extent of effective volume reduction did not differ significantly with respect to the two types of treatment.

Kidney patients and echocardiography

Before starting the two renal replacement therapies, there was no significant difference in the left atrial cross diameter, but by the end of the HDF, a significant shortening (HDF: 45.43 ± 5.2 mm vs. 40.77 ± 6 mm, $p = 0.000166$) was detected. Although the left atrial cross diameter decreased during HD, too, this change did not reach a significant extent ($p = 0.11$).

There was no significant change in the left ventricular ejection fraction or the left ventricular mass index before and after the treatment (EF HD: 56.57 ± 9.2 % vs. 56 ± 7.7 %; EF HDF: 56.47 ± 8.7 % vs. 54.57 ± 6.8 %; left ventricular mass index HD: 211 ± 69 g/m^2 vs. 193 ± 64 g/m^2; left ventricular mass index HDF: 188 ± 62 g/m^2 vs. 185 ± 65 g/m^2). The left ventricular end-systolic and end-diastolic diameters did not change significantly, either. These variables did not show any significant correlation with any of the parameters examined.

The Holter ECG findings of our kidney patients

Although supraventricular premature beats manifested themselves more frequently during hemodialysis, there was no statistically significant difference between the two renal replacement modalities (p = 0.14). Although no atrial fibrillation was detected in the case of our patients at any stages of the examinations, the number of supraventricular premature beats was frequent (HD: 363 beats/24 hours; HDF: 350 beats/24 hours).

Correlations of atrial electrocardiographic markers and echocardiographic parameters

During hemodiafiltration we found a positive correlation between the starting left atrial diameter and the number of supraventricular premature beats (r = 0.4556, p = 0.011). Interestingly, during hemodiafiltration, the shortening of the left atrial cross diameter negatively correlated with the frequency of supraventricular premature beats (r = -0.43; p = 0.016).

Changes in heart rate and blood pressure in the course of renal replacement therapies

We characterized changes in heart rate by the length of the section between the peaks of two consecutive R-waves (known as the RR-cycle length). At minute 30 of hemodiafiltration the RR-cycle lengthened compared to the value measured at the start (852 ± 104.3 msec; p <0.05) – indicating a temporary fall in heart frequency – after which it shortened once again. In the course of hemodialysis, RR-cycle length shortened in the 2nd hour after the end of the treatments compared to the start (minute 0: 800.6 ± 96.2 msec; 2 hours after treatment: 746.6 ± 125.5 msec; p <0.05).

With reference to blood pressure it can be established that in the case of both types of treatment a fast fall in blood pressure occurred after the start, which was significant at minute 15 already (p <0.001). After that, systolic blood pressure did not change significantly in any of the cases, while the diastolic values showed a slight rise after the end of the treatments. In the case of the convective therapy, we observed heightened systolic and diastolic values at the beginning of the treatment already (HDF: systolic 152 ± 24 mmHg, diastolic 83 ± 19 mmHg; HD: systolic 144 ± 22 mmHg, diastolic 79 ± 14 mmHg), but this difference was not statistically significant with respect to the two treatment modalities.

The clinical consequences of anxiolytic withdrawal in relation to the analysis of one of our cases

In January 2014, a 55-year-old female patient was admitted to the Emergency Department of our Clinic with the following symptoms: paroxysmal hypertension, headache, vertigo, tachycardia, epiphora, nausea as well as varying states of consciousness during the attacks. The patient's medical history included Caesarean section, (1984); abdominal surgery due to mechanical ileus, laparoscopic cholecystectomy (1995); total thyroidectomy due to gastroesophageal reflux disease and benign non-toxic multinodular goitre and hormone substitution due to hypothyroidism. In 2008, based on heightened fasting blood sugar levels, type 2 diabetes mellitus was diagnosed. The patient first experienced attacks involving a rise in blood pressure and sinus tachycardia in 2003, because of which she underwent tests several times but no clear organic reason was identified; cardiac arrhythmias or ischemic heart disease were not confirmed. The possibility of endocrine diseases – especially pheochromocytoma – was excluded several times. There were no intracranial tumour, cerebrovascular ischemia/bleeding or epilepsy confirmed underlying the deteriorating consciousness observable during the hypertensive episodes. After several diagnostic procedures in 2004, panic syndrome was assumed; anxiolytic and antidepressant therapy was started. In the course of the psychiatric follow-up examinations a fall in the number of attacks was confirmed but in 2013, the earlier therapy was discontinued due to drug addiction. Thereafter, for approximately 1 year, the patient was symptom and complaint free. When admitted to our clinic, she took the following drugs: metoprolol 2x100 mg, esomeprazole 40 mg/day, levothyroxine 100 ug/day, allopurinol 100 mg/day, rapid-acting insulin 3 times a day, long-acting insulin once a day.

In order to precisely clarify the reasons underlying the attacks, patient consulted the Division of Rare Diseases of our Clinic in 2014. While waiting, she developed malaise with blood pressure of 230/100 mmHg, a heart rate of 160-180/minute and the loss of consciousness. On patient's left cheek we also observed focal muscle vibration and epiphora. Considering patient's unstable condition we continued her observation and treatment at the Intensive Care Unit of our Clinic, where the attack spontaneously resolved in a few minutes without medical treatment and her condition rapidly improved. After the attack terminated, patient's heart rate fell to 90/minute and her blood pressure normalised spontaneously.

After the crisis, no arrhythmia, neurological deficit or other significant clinical abnormalities were detected. In the first week of patient's observation, these attacks occurred approximately 2-4 times a day. The respective hypertensive crises and the associated sinus tachycardia attacks lasted cca. 3-5 minutes and resolved spontaneously, without pharmacological intervention. Between two attacks the patient was absolutely symptom and

complaint-free. Because of the accumulating attacks we administered a combination of α-receptor-blocker (doxazosine 4 mg per day) and β-receptor blocker (bisoprolol 2x5 mg per day), as the effects of which the maximum blood pressure value and maximum heart rate dropped, but there was no meaningful change in the frequency of attacks. On the 12-lead surface ECG and the 24-hour Holter ECG we recorded, we registered the sinus tachycardia occurring during the attack but did not detect any other atrial or ventricular arrhythmia.

In order to clarify the aetiology of the above syndrome we performed several tests. On the basis of renal Doppler ultrasound we excluded the possibility of renovascular origin. In both kidney arteries we measured physiological flow; there was no significant difference (0.6 vs. 0.7) between the resistance indices (RI) of the two sides. Even though the endocrine diseases causing malignant hypertension had earlier been excluded, the patient underwent new tests with respect to pheochromocytoma and carcinoid. On one occasion we measured higher chromogranin A level, but this finding was confirmed to be false positive because at the time of collecting the sample the patient turned out to have taken a proton pump inhibitor (after omitting which the sample contained physiological chromogranin A level).

Surprisingly, the CT examination showed a pattern characteristic of adenoma in the left adrenal gland. At the same time, normal levels of 5-hidroxi-indol acetic acid, metanephrine, normetanephrine and dopamine were measured in the urine. On one occasion, in the blood sample taken during an attack we found slightly increased noradrenalin and dopamine concentration, which, however, fell short of the criteria of pheochromocytoma.

Finally, based on the ^{131}I-MIBG scan, the possibility of pheochromocytoma could be totally excluded.

On the basis of the laboratory tests we performed, we also excluded the possibility of endocrine abnormalities causing other, paroxysmal hypertension (hyperaldosteronism, adrenal adenoma). Our patient's thyroid hormone test findings were physiological; due to earlier thyroidectomy she was administered constant hormone replacement. After excluding the above endocrine medical conditions our attention turned to anxiolytic therapy. Following psychiatric examination we restarted the administration of the earlier administered alprazolam in a dose of 1 mg (2x0.5 mg) a day. After that the patient's condition showed improvement and the daily doses of α- and β- blockers could be reduced. Later, as alprazolam caused sleepiness and tiredness, its dose was halved to 0.5 mg but, due to the reappearance of hypertensive episodes we were forced to raise the dose to 1 mg per day once again.

Patient spent a total of 4 weeks at the Intensive Care Unit of our Clinic. At the time of her discharge from hospital, while her condition had significantly improved, she still suffered from minor malaise.

The relationship between pseudopheochromocytoma caused by anxiolytic withdrawal and neurogenic cholecystokinin

In case no straightforward organic reason can be detected underlying the hypertensive episodes occurring in attacks and there is no evidence for the presence of pheochromocytoma, the possibility of pseudopheochromocytoma should be considered. It is important to note that the symptoms developing in such cases should not be put down to emotional stress but to the *excessive dominance of the sympatho-adrenal vegetative nervous system*. Although anxiety symptoms can be discovered in the case of some patients, these are usually triggered by the fear of the attacks. It may be misleading that blood pressure rise can be observed in the course of a regular panic attack, too, but it is much milder than in the case of pseudopheochromocytoma.

Benzodiazepines (thus including alprazolam) are widely known to have anxiolytic, sedative, hypnotic, anticonvulsive and muscle relaxing effects. They exercise their effects via the $GABA_A$ receptor. $GABA_A$ receptor is an ionotropic receptor, a ligand-dependent Cl^- channel. The endogenous ligand is the GABA itself, which is one of the inhibiting neurotransmitters of the central nervous system.

In addition to the above, the role of the *neurogenic cholecystokinin* (CCK) receptor is also assumed in the development of the medical condition. The activation of the CCK receptor has been proven to play a role in the development of fear and panic. The discontinuation of benzodiazepine administration involves the lifting of the inhibition of CCK-receptors, whereby the vascular tone increases which, in turn, causes paroxysmal rise in blood pressure, and tachycardia.

On the basis of all the above it can be established that the sudden discontinuation of anxiolytics may lead to this serious clinical medical condition through the co-emergence of several pathogenic processes. In the background underlying the leading symptoms (sudden hypertension and altered atrial impulse generation), the pathogenic roles of sudden changes in the concentration of vegetative nervous system mediators and the consequentially enhanced sympathetic nervous system tone can be assumed.

Discussion

Atrial arrhythmia vulnerability and renal replacement therapy

The structural and electrophysiological heterogeneity of the myocardium plays an important role in the development of regional depolarization and/or repolarization inhomogeneity, as a consequence of which parts may develop in the cardiac muscle that are able to conduct electrical impulse only in a certain direction (known as unidirectional block). This phenomenon facilitates arrhythmogenic mechanisms like, among others, reentry and premature beats. Unsurprisingly, these pathogenic processes involve an increased danger of the development of both atrial and ventricular arrhythmias.

In kidney patients there is an increased frequency of the above arrhythmia mechanisms and the renal replacement therapy itself, too, may play a role in this unfavourable process. Studies in recent years have shed light on the fact that the respective kidney replacement modalities influence the mortality indicators of end-stage kidney patients in different ways. It has been revealed that, compared to traditional hemodialysis, hemodiafiltration based on the principle of convective transport reduces cardiovascular mortality by 35 %. In the background underlying the favourable statistical data observed in hemodiafiltration, the more effective clearance ensured by the convective transport, affecting toxins of small and medium large molecular weight, the use of what are referred to as high-flux membranes and the optimisation of the convection volume all play a role. In the course of hemodiafiltration the concentration of acute phase proteins does not rise and, as a consequence of the more efficient removal of β_2-mivcroglobulin, the incidence of amyloidosis, too, falls by approximately 50 %. It is known that, during hemodiafiltration, oxidative stress falls, anaemia requiring eritropoetin becomes less frequent, while kidney patients' lipid profile may improve. Considering all these favourable characteristics it has been recently suggested that the favourable effects provided by the convective transport may slow down the progress of cardiac target organ damage and reduce arrhythmia-related mortality.

Considering all these advantages experienced as well as data in the technical literature in relation of hemodiafiltration, our working group set out to examine the effects of convective therapy and traditional hemodialysis on propensity to atrial arrhythmia.

Several examinations have been undertaken in the past decades in order to be able to predict atrial fibrillation. Based on the findings of the studies so far it can be established that in the case of patients suffering from paroxysmal atrial fibrillation, in the course of sinus rhythm, the duration and dispersion of the P-wave may be prolonged, which may be a reliable predictor of another arrhythmia episode.

Our current examinations proved that in the course of hemodiafiltration the atrial electrocardiographic parameters did not change significantly. During hemodialysis we experienced significant prolongation, on the other hand, with respect to the arrhythmia markers examined. The background of these results has not been clarified completely yet but the difference in the distribution of intracardiac, intravascular and interstitial fluid spaces between the two renal replacement modalities arises as an influencing factor. The different behaviour of fluid compartments is also confirmed by the significant difference observed when measuring the left atrial cross diameter. The atrial wall tension occurring as a consequence of hemodiafiltration, which eases to a greater extent, and the reduced atrial arrhythmia vulnerability may serve as explanations for the lower frequency of the number of atrial premature beats, which also foreshadows the lower chance of the development of atrial fibrillation. It is important to emphasise that all these differences developed while there was no difference with respect to the effective volume removal of the two renal replacement modalities. We observed no close correlation with respect to the potassium-, magnesium-, phosphate-levels or atrial markers, so in the course of our current examinations we were unable to prove the role of these electrolytes in the arrhythmogenesis. The close correlations observed with reference to the atrial ECG parameters and the sodium- and calcium levels suggest that the changes in concentration affecting these ions and the suddenly developing imbalances may facilitate the occurrence of atrial arrhythmias constituting a danger in the course of the renal replacement treatment method to a great extent. The role of electrolytes in arrhythmogenesis, not clarified precisely yet, is also underpinned by our finding that the bicarbonate concentration measured in the beginning of the renal replacement therapy showed a significant negative correlation with the atrial arrhythmia markers during hemodialysis. According to our data we can conclude that the close monitoring of the electrolytes specified above, the ion profiling applied in the clinical practice, may contribute to reducing propensity to atrial arrhythmias during the application of the respective renal replacement therapies.

On the basis of the findings of the 24-hour ECG monitoring we can conclude that although no atrial fibrillation was detected during the application of either modalities, the number of atrial premature beats was much more frequent during hemodialysis, which indicates an increased arrhythmia vulnerability during the conventional therapy and at the same time implies the beneficial effect of hemodiafiltration serving arrhythmia prevention.

Our findings observed with respect to the different forms of kidney replacement, explaining the advantages of convective transport, may be put down to the different distribution of fluid spaces and the dissimilarities arising from the differences in intracardiac/intravascular pressure conditions. At the same time, both the more efficient detoxification capacity of

hemodiafiltration and the advantages arising from this, already proven on the basis of earlier international examinations, are most probable to have played a role in the development of the differences observed in the course of our current study.

Conclusions from the consequences of anxiolytic withdrawal

Several clinical medical conditions may cause paroxysmal hypertension. If the pathogenic process develops as a consequence of enhanced sympathetic nervous system tone, hypertension usually couples with a rise in the frequency of cardiac impulse generation. In the case of pseudopheochromocytoma, the frequency of α_1- and β-receptors grows, which is well proven also by the clinical observation that people suffering from this disease usually react well to α- and β-receptor blocking therapies. At the same time, in certain cases these medications do not trigger the expected therapeutic effect, in which cases complementary psychotherapy (both psychological and pharmacotherapeutic care) may bring success to the treatment. The efficiency of combined treatment is also supported by the study in which additive therapeutic advantages were reported in relation to psychotherapy applied in addition to the co-administration of certain antidepressants (desipramin, paroxetin) and anxiolytics. It is important to note at the same time that these treatment methods are only able to influence the symptoms and the extent of instability of the vegetative nervous system but are unable to cure the disease itself.

Governed by the desire to understand the mechanism of pseudopheochromocytoma causing a rapid deterioration of condition and to improve its treatment efficiency, in the second part of our study we demonstrated the role of autonomic dysregulation in the development of this serious clinical syndrome through the medical history of one of our female patients. The patient discontinued taking alprazolam, a benzodiazepine with anxiolytic effect she had been previously ordered to take for her panic disease. As a consequence of the discontinuation of the therapy, autonomic instability and sympathetic activation developed, which caused serious paroxysmal hypertension, cerebrovascular disorder and sinus tachycardia. It must be emphasised that all these were not the consequences of the panic disease and the malaise was not provoked by psychological strain. After secondary hypertension was safely excluded, the diagnosis of pseudopheochromocytoma became straightforward. The initially introduced α- and β- receptor-blocking pharmacological treatment did not have the expected therapeutic effect; paroxysmal episodes involving serious deterioration of condition continued to follow. It was then that we realised that the withdrawal of alprazolam could have a role in the development of the clinical syndrome. After the benzodiazepine treatment was restarted, the patient's clinical condition and quality of life showed rapid improvement. When the temporary reduction of the

dose of alprazolam involved a rise in blood pressure as well as propensity to tachycardia (the positive challenge test often applied in pharmacology), the pathogenic role of the benzodiazepine withdrawal was once again confirmed. As far as we are informed, our case was the first one in the technical literature to draw attention to the serious clinical consequence of anxiolytic withdrawal and at the same time confirmed the cardinal regulatory role of the change in autonomic tone (sympathetic activation) in blood pressure control and cardiac impulse generation. In the background underlying the rapid deterioration in our patient's medical condition we identified, as a potential mechanism, *the activation of the cholecystokinin receptor*, which is known to show a correlation with the genesis of fear and panic. The reason is that, as a consequence of the sudden withdrawal of benzodiazepine the cholecystokinin receptors are released from inhibition, thereby causing sympathetic nervous system activation, which may be the explanation for the emergence of this serious clinical syndrome. It can be established that, consistently with the data in the technical literature about the treatment of pseudopheochromocytoma, our observations, too, confirmed that in addition to the application of α- and beta-receptor blockers, a timely and tailor-made psychological and psycho-pharmacological therapy, too, constitutes an important element of curing this disease.

Summary

1. During hemodiafiltration, the P-wave duration and dispersion did not change significantly, while in the case of conventional hemodialysis these atrial arrhythmia markers showed significant prolongation.

2. During hemodialysis atrial premature beats occurred more frequently, even though to an insignificant extent, than in the case of hemodiafiltration. This result implies the enhanced role of conventional hemodialysis in the development of atrial arrhythmias.

3. The P-wave duration and dispersion and the significant correlation observed with respect to serum sodium and calcium, the concentration changes affecting these ions, draw attention to the pathogenic role of the former in atrial arrhythmogenesis.

4. The significant decrease of the left atrial cross diameter in the course of hemodiafiltration may follow from the difference of the distribution of fluid spaces between the two types of renal replacement therapies.

5. We have identified the activation of the cholecystokinin receptor, which is known to show a correlation with the genesis of fear and panic, as a potential mechanism of pseudopheochromocytoma. The sudden withdrawal of benzodiazepine, causing the release of cholecystokinin receptors, may trigger, through the sympathetic nervous system activation, a rise in blood pressure and enhanced propensity to arrhythmias.

6. In the case of pseudopheochromocytoma the rise in blood pressure and the propensity to tachycardia as consequences of the temporary decrease of the dose of alprazolam (the positive challenge test often applied in pharmacology) confirmed the pathogenic role of benzodiazepine withdrawal.

7. On the basis of our observations regarding the treatment of pseudopheochromocytoma it can be established that in addition to the application of α- and beta-receptor blockers, a timely and tailor-made psychological and psycho-pharmacological therapy, too, constitutes an important element of curing this disease.

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List of publications related to the dissertation

1. **Páll, A.**, Czifra, Á., Sebestyén, V., Becs, G., Kun, C., Balla, J., Paragh, G., Lőrincz, I., Páll, D., Padra, J. T., Agarwal, A., Zarjou, A., Szabó, Z.: Hemodiafiltration and hemodialysis differently affect P wave duration and dispersion on the surface electrocardiogram.
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2. **Páll, A.**, Becs, G., Erdei, A., Sira, L., Czifra, Á., Barna, S., Kovács, P., Páll, D., Pfliegler, G., Paragh, G., Szabó, Z.: Pseudopheochromocytoma induced by anxiolytic withdrawal.
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List of other publications

3. **Páll, A.**, Sebestyén, V., Czifra, Á., Becs, G., Anupam, A., Zarjou, A., Paragh, G., Lőrincz, I., Balla, J., Szabó, Z.: The disparate effect of hemodialysis and hemodiafiltration on T-wave-peak-to-end interval (Tpe) and Tpe/QT ratio of the surface electrocardiogram.
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IF: 1.52

Total IF of journals (all publications): 9,092
Total IF of journals (publications related to the dissertation): 3,059

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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