ASSESSMENT OF NEURONAL DYSFUNCTION IN PATIENT GROUPS WITH HIGH RISK OF NEUROPATHY

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Summary of PhD Thesis

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Relevant publications

Full papers

I**. Magony S,** Nyiraty Sz, Tóth B, Pesei F, Orosz A, Ábrahám Gy, Kempler P, Lengyel, Cs, Várkonyi T. Peripheral sensory nerve hyperaesthesia in women with polycystic ovary syndrome MINERVA ENDOCRINOLOGY 2021 **(impact factor: 1.529)**

II. **Magony S,** Nyiraty Sz, Fehértemplomi K, Tóth B, Pesei F, Orosz A, Lengyel Cs, Kempler P, Várkonyi T. Inzulinpumpa-kezelést igénylő, kedvezőtlen anyagcsere-állapotú 1-es típusú diabeteses betegek autonóm idegrendszeri funkciójának jellemzői DIABETOLOGIA HUNGARICA 2020;28(6)289-296.

III. **Magony S,** Nyiraty Sz, Fehértemplomi K, Tóth B, Pesei F, Orosz A, Lengyel Cs, Kempler P, Horváth V, Várkonyi T. Long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump. DIABETES STOFFWECHSEL UND HERZ 2021;30(3)193-197. (**impact factor 0.262)**

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1 Introduction and aims of the study

The aims of the preventive medicine are protecting, promoting, and maintaining the state of well-being and health, avoiding disease, disability, and death on individuals and on the populations. The preventive medicine through interdisciplinary ways approaches many factors influencing the patients' health. Neuropathy is one of the most detrimental and diversified neurological conditions that damages the balance of several physiologic processes. It considerably impairs patients' quality of life and also associated with an increased morbidity and mortality. Preventing the manifestation or the progression of neuropathy is essential in high-risk patients. Therefore, it is very important to explore all possible conditions which might lead to this complication. In addition to high blood glucose, there are a number of pathological conditions which may potentially cause neuropathy. Common risk factors are proven of distal symmetric sensorimotor polyneuropathy in diabetes and cardiovascular diseases including age, hypertension, dyslipidaemia, oxidative stress and obesity. Cardiovascular autonomic neuropathy (AN) is also commonly associated with well-known macrovascular risk factors in type 1 and type 2 diabetic patients including high blood pressure, hypertriglyceridemia and smoking. In accordance with the findings indicating that severe hyperglycaemia is not the exclusive cause of the neuronal damage, both autonomic and sensory neuropathies were documented in patients with prediabetes. The prevalence of polyneuropathy in prediabetes is associated with obesity, visceral fat and peripheral arterial disease. Higher insulin levels exert a detrimental effect on the neuronal systems, as hyperinsulinemic/euglycemic clamps revealed increased muscle and cardiac sympathetic activity in healthy subjects, and an altered response to orthostatic stress in older adults with type 2 diabetes. In a cross-sectional study on 2,035 patients with metabolic syndrome, peripheral neuropathy was associated with insulin resistance independently of the additional components of metabolic syndrome. Insulin resistance has a decisive role in the pathogenesis of polycystic ovary syndrome (PCOS), and it is associated with a high number of risk factors for the development of neuropathy, including impaired glucose metabolism, hypertension, obesity and hyperlipidaemia. In addition, the excess of androgens in women with PCOS might be a contributing factor in the development of cardiovascular diseases and probably neuropathy as well. Although PCOS is the most common endocrine disorder in females, there is a very limited amount of data available on the manifestation of neuronal dysfunctions in the patients. Based on the lack of the previous characterization of the neuronal systems in PCOS, we designed a study as a first part of this

thesis with the aim to the assess the cardiovascular autonomic and peripheral sensory functions in women with this disease.

It is widely accepted that in type 1 diabetes AN is an independent risk factor for mortality, that particularly explained by the relatively increased activity of the sympathetic nervous system due to an attenuated parasympathetic function. Further etiologic factors of the poor life expectancy in patients with AN are the impaired cardiovascular adaptation, the development of diastolic dysfunction and the increased rate of arrhythmias that may be associated with sudden cardiac death or respiratory arrest. As AN is a critical determinant of the cardiovascular state, it is mandatory to apply all the possible therapeutic options to prevent the development or to reduce the progression of this neuronal complication. In type 1 diabetes, one of the most important tool to achieve these aims is to keep the glycemic control strictly in the target range. It is not known how long it takes for metabolic optimization to have a beneficial effect on the functioning of the autonomic nervous system. It is a well-known fact, that an association exists between long-term glycemic control and the incidence of neuropathy, as it was proven in several previous studies on high number of patients. In previous study the observations underline the importance of the role of the early glycemic control in the long-term progression of AN and proves the dominance of the parasympathetic impairment in the initial phase of AN. A metaanalysis of 17 randomized trials revealed, that the effective glucose control significantly prevented the development of clinical neuropathy in type 1 diabetes mellitus, whereas this association in type 2 diabetes is less evident. No doubt that the long-term glycemic control plays an important role in the development of neuropathy in type 1 diabetes, but some remarkable observations in small number of patients suggest that even short-term changes of glucose levels may also affect the nervous system function in both types of diabetes or even in healthy subjects. The data from the literature clearly suggest that assuring a well-treated glucose metabolism is the only way to prevent the development or to reduce the progression of neuropathy. The most intensive form of the treatment and optimization of the glycemic control in type 1 diabetes is the introduction of subcutaneous pump therapy which ensures the continuous insulin supply by the basal rate of the administration supplemented with bolus doses before main meals. Analyzing the previous articles that was not clear what time should be elapsed in better glycemic condition to achieve an improvement of the neuronal function. The available data from small studies extend from hours to years defining the glycemic condition affecting the parameters of neuropathy. In the second part of this thesis we explored the characteristics of autonomic cardiovascular function in those type 1 diabetic patients whose glycemic control necessitated insulin pump therapy.

Based on previous data and our preliminary assumptions discussed above our main goals were:
- to assess the peripheral sensory and the cardiovascular autonomic function in women with polycystic ovary syndrome,
- to explore the association of the measured neuronal functions and the possible risk factors of neuropathy (age, BMI, serum insulin, blood glucose, HOMA index, testosterone, androstendione),
- to characterize the nature of the cardiovascular autonomic dysfunctions in type 1 patients at the introduction of their insulin pump therapy,
- to follow the cardiovascular autonomic functions in type 1 diabetic patients with short-term insulin pump treatment (2 months),
- to describe the long-term trends (6 years) of alterations in the cardiovascular autonomic reflexes in type 1 diabetic patients with insulin pump treatment.

2 Patients

2.1 Assessment of neuronal function in women with polycystic ovary syndrome

27 women with PCOS were involved in the study (age: 28.7±1.8 years, mean±SE). Fasting blood glucose, fasting serum insulin, testosterone, androstenedione, body weight and height were measured in PCOS patients, and in controls with the exception of serum insulin levels. Homeostasis Model Assessment (HOMA) was expressed from fasting insulin and glucose values in PCOS patients. None of the patients had been diagnosed with diabetes; fasting blood glucose was 4.5±0.09 mmol/L., the mean BMI of the patient group was 29.7 kg/m2, the patients had no symptoms of neuropathy. 24 healthy women with normal weight were the controls, who had no significant differences in comparison to the patient group in the descriptive parameters except for BMI (age: 28.1±1 years, fasting glucose: 4.1±0.07 mmol/L, BMI: 22.6±0.8 kg/m^2). The HOMA index of the PCOS group indicated insulin resistance (2.64±0.59 vs 1.92±0.33, $p<0.05$; PCOs vs control). PCOS was diagnosed following the Rotterdam consensus.

2.2 Short-term follow-up of the cardiovascular autonomic function among patients with type 1 diabetes treated with insulin pump

38 (23 women and 15 men) patients with type 1 diabetes and 10 healthy controls (6 women and 4 men) participated in this study. Patients were young adults (29.5±1.3 years±SE), the duration of diabetes at baseline was 13.8±1.5 years. Their mean BMI was 23.2±0.6. Before the pump treatment the blood glucose fluctuations in all patients were hectic, often exceeding 10 mmol / l / day (24/38 patients). The HbA1c was higher than 8% in all cases (38/38 patients), their mean HbA1c was 8.7%±0.2. The incidence of complications associated with diabetes was detected as 16 patients with retinopathy, 10 patients with nephropathy, and 10 patients with confirmed diabetic neuropathy. The first autonomic test was performed in all patients within 1 week before the start of their insulin pump therapy. The second autonomic function test was performed 2 months after the initiation of pump treatment. Patients were tested for HbA1c at the beginning of pump treatment and 2 months later. The control group did not differ significantly from the patient group in terms of descriptive parameters (mean age: 27.8 ± 2 years, mean BMI: 24.1±0.4).

2.3 Long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

This study involved 13 patients (7 women and 6 men) with type 1 diabetes. The patients were young adults (30.4±2.7 years, mean±SE), the duration of diabetes at baseline was 16.5 ± 2.7 years. The BMI of the patient group at the initiation of the pump treatment was 24.2±1.0 kg/m2, the HbA1c was 8.85±0.2% at the same time. The first cardiovascular reflex tests were performed in all patients within 1 week before starting their subcutaneous insulin pump therapy. The assessments of the cardiovascular autonomic function were performed after 2 months and 6 years of the pump treatment. The HbA1c levels were determined at all of the three tests.

3 Methods

3.1 Assessment of AN

The presence and severity of AN was characterized using the four standard cardiovascular reflex tests (CRT) in all patient [31]. These measurements provide a non-invasive, clinically relevant, reproducible and standardized gold-standard assessment of the autonomic function Three of these tests record the changes in heart rate during specific manoeuvres, while the fourth

test is designed to monitor blood pressure changes. Most of the tests aiming to detect heart rate changes are used primarily but not exclusively for the assessment of parasympathetic innervation, while the blood pressure response predominantly indicates the impairment of sympathetic functions. The heart rate changes were analyzed during deep breathing, in positions of lying and standing up (30/15 ratio) and during and after of Valsalva manoeuvre. Systolic blood pressure was determined in response from lying to standing up. Finally each CRT was scored as 0 (normal), 1 (borderline) or 2 (abnormal) and by this method an autonomic score (0-8) was calculated to express the overall severity of AN. Age-corrected normal reference values were applied based on the definition of Ewing and the recommendations of the Toronto Neuropathy Expert Group.

3.2 Assessment of peripheral sensory neuropathy by application of Neurometer

The peripheral sensory function was studied with a Neurometer (Neurotron Incorporated, Baltimore, MD, USA). This device is intended to quantify the function of different nerve fibres and provides a simple, non-invasive, and quantitative measure of peripheral sensory function. Low voltage electric sine wave stimulation was applied transcutaneously on the upper and lower extremities, and the current perception threshold (CPT) values were determined. The sensory function of the median and peroneal nerves was tested in our study. The surface electrodes, 1 cm in diameter, were placed on the terminal phalanx of the index and the great toe. The electrodes were fixed only on intact skin surface, because wounds or scars would have disturbed the peripheral sensations. The amplitude of the delivered stimuli was between 0.01 and 9.99 mA. The stimulus was initially increased until a sensation was reported, then short stimuli (2 to 5 s) were applied at progressively lower amplitudes until a minimal threshold for consistent detection was determined. The CPT values of the upper and lower limbs were detected at three different stimulating frequencies (2 kHz, 250 Hz, and 5 Hz).

4 Results

4.1 Results of the cardiovascular autonomic and peripheral sensory functional tests in women with polycystic ovary syndrome

The statistical evaluation revealed no significant differences in the four standard CRT

reflecting the parasympathetic and sympathetic cardiovascular function (heart rate responses to deep breathing: 24.9±1.9 vs 24.5±1.6 beats/min; Valsalva ratio: 1.68±0.07 vs 1.86±0.06; 30/15 ratio: 1.4±0.06 vs 1.49± 0.06; orthostatic systolic blood pressure drop: 2.5±0.8 vs 2±0.8 mmHg, mean±SE, PCOS vs control; $p>0.05$ respectively).

The CPT levels in the median nerve at all three testing frequencies were significantly lower in the PCOS patients than in the controls. Comparison of the CPT values in the peroneal nerve yielded similar findings, as these were significantly lower than in the control group. To explore the possible role of insulin resistance in the abnormal sensory function, correlation tests were performed between HOMA indexes and the CPT values. No associations were observed between the CPT values measured in the median or the peroneal nerves, and the degree of insulin resistance expressed by the HOMA index. Most of the analyses revealed no significant correlations between the BMI of PCOS patients and the CPT values measured in any of the extremies. The only exception was a significant negative correlation between BMI and the CPT in the peroneal nerve at 5 Hz stimulation ($r = -0.39$; $p<0.05$). There was no statistical association between testosterone or androstenedione levels and the measured parameters of neuropathy. Multiple linear regression analysis yielded no predictors for sensory hyperaesthesia from the measured parameters (age, BMI, serum insulin, blood glucose, HOMA index, testosterone, androstendione).

4.2 Results of the study of short-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

The duration of type 1 diabetes at the time of the pump treatment initiation correlated strongly with the score for the overall severity of AN ($r=0.51$, $p<0.05$). The positive correlation suggests that the overall rate of AN is more severe with longer duration of diabetes with poor metabolic conditions.

The duration of diabetes and the result of the heart rate response to respiration test significantly correlated at the implementation of pump therapy. The correlation is negative, as the lower the rate in heart rate response, associated with the longer the duration of diabetes ($r=-0.63$, $p<0.001$).

During the 2-month follow-up, AN score slightly significantly decreased. The baseline value of the AN score in the diabetic patients' group was significantly higher than in the controls (AN score: 2.2±0.2 vs 0.9±0.1, $p<0,05$). The AN scores measured 2 months later in insulin pump-treated diabetic patients did not differ significantly from the healthy subjects (1.5±0.2 vs

0.9±0.1, p>0.05).

The heart rate response to deep breathing increased significantly during the two months of the pump treatment (18.6±2,1 vs 22.4±2 beats/min, p<0.05), suggesting an improvement in parasympathetic function. As in the case of the AN scores, the heart rate responses to deep breathing in diabetic patients were significantly lower than in the controls, while there was no difference from controls after 2 months of insulin pump treatment (baseline: 18.6±1.5 vs 32.6±3.8 beats/min., p<0.001, follow-up: 22.4±2 vs 32.6±3.8 beats/min p>0.05).

4.3 Result of the study of long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump

At baseline a moderate severity of autonomic neuropathy was revealed, as we observed it in the study of short-term follow-up of the autonomic function among patients with type 1 diabetes newly treated with insulin pump. During the follow-up an improvement of the total autonomic score was detected two months after the implementation of pump (2.85±0.3 vs 1.23±0.3, p<0.01). The AN score measured six years later was identical to the initial value (2.85±0.3 vs 2.85±0.4 p>0.05**)**. The four standard CRT did not differ significantly during the follow-up. In 3 of the 4 tests there was a non-significant tendency of an improvement by the second month, while a progression was not revealed by the 6th year in comparison to the initial values at any of the tests.

5 Discussion

The best way to prevent the development or the progression of neuropathy is to seek for it in patients with high risk. This is the most effective way to intervene the pathogenetic process in time in case of positive results. We have chosen two groups with pathogenetic conditions that responsible for the development of neuronal dysfunction. We decided to analyze patients with the potentially early and patients with presumably advanced forms of neuropathy, this is the explanation why we included patients with PCOS and long-standing type 1 diabetes with poor glycemic control. Moreover, the theoretically benefical effect of insulin pump treatment was also studied in the latter group.

Our data revealed that heart rate and blood pressure responses in PCOS patients and healthy control subjects are similar. The putative harmful effect of insulin resistance, hyperandrogenism

or obesity did not lead to a deficit in the parasympathetic or the sympathetic regulation in patients with PCOS. As the mean age of the patients was 28 years, they were probably too young to be seriously exposed to the effects of insulin resistance exerted on autonomic function. It is very difficult to determine the duration of insulin resistance in PCOS. Moreover, in these patients the degree of insulin resistance may not have reached the threshold of sensitivity of the autonomic system, as the mean value of the HOMA index was only slightly higher than the previously described normal range. This is the first study to evaluate data of the cardiovascular reflex tests of PCOS patients, and the findings indicate intact autonomic functions. Our data support a hypothesis that in young PCOS patients, insulin resistance and the additional components of the syndrome without diabetes do not exert a negative effect on autonomic function beside the potential risk.

The evaluation of the sensory function revealed characteristic new findings in these patients. In both extremities and at all stimulating frequencies, the women with PCOS consequently perceived the electric stimulation at lower intensities than the healthy controls. As their threshold of perception was lower, they presumably became more sensitive to various stimuli than the healthy women. The Neurometer, our method of choice allows to selectively stimulate three types of the sensory nerve fibres. We tested the large and small myelinated fibres at 2000 Hz and 250 Hz, the sensory conduction of the small unmyelinated fibres was determined at a testing frequency of 5 Hz. In accordance with the detected lower perception thresholds we found that in the large and small sensory fibres of the median as well as the peroneal nerves, hyperaesthesia was present in women with PCOs. Several aspects of these findings provide new approach to the pathophysiology of peripheral nerve damage. The described hyperaesthesia was also found in a minority of patients with prediabetes or diabetes at a very early stage of neuropathy, or it is associated with painful symptoms of neuropathy. The nature of the progression of sensory neuropathy is still a subject of debate in the literature, but it is widely accepted that hyperaesthesia is a very early and mostly silent manifestation of neuropathy, which quickly turns into hypaesthesia as neuropathy worsens. Most of the patients are tested in the late phase of hypaesthesia, as this is the period when the symptoms appear, and patients seek medical aid. Hyperaesthesia is not a unique form of very early diabetic neuropathy. This neuronal complication has been proven in primary biliary cirrhosis with autoimmune pathogenesis. In our PCOS patients, lower perception thresholds were not associated with painful neuropathy, as our subjects did not present with any symptoms. The pathogenesis of the observed sensory abnormality might be partially explained by obesity, as higher BMI correlated significantly with lower perception thresholds in the peroneal nerve at

the 5 Hz stimulating frequency. The same phenomenon was observed earlier in a population-based study without any symptoms or signs of polyneuropathy. Hyperaesthesia in our patients with insulin resistance might share a common pathogenesis with the well-known phenomenon of incipient hyperfunctions, as in the case of characteristic very early manifestations of increased microvascular circulation in the retina precedes the clinical manifestations of retinopathy, while hyperfiltration is an early characteristic sign of nephropathy. Hyperaesthesia in the early stage of neuropathy might be also considered to be an enhanced physiologic function in the course of the developing complication. Our observations point to a generalized early alteration without a selectivity to site or function in patients with PCOS and underlines the magnitude of seeking for neuronal dysfunctions in patients without symptoms but with high risk.

The concept of the short-term and long-term follow-up of the autonomic function among patients with type 1 diabetes treated with insulin pump based on the well-known fact that an unfavorable metabolic situation increases the risk of developing autonomic neuropathy. The prevalence of parasympathetic autonomic neuropathy was significantly correlated not only with HbA1c but also with a number of other parameters, including the duration of diabetes. In the case of all type 1 diabetic patients in our study, despite previous therapeutic efforts, a stable metabolic state could not be achieved, so it became necessary to introduce insulin pump therapy.

We had the opportunity to follow type 1 diabetic patients whose glycemic control necessitated the application of a subcutaneous insulin pump treatment. In our study, a significant association was found at baseline between the duration of the disease expressed in years and the severity of autonomic neuropathy, suggesting that both prevalence and the degree of neuropathy are related to the length of chronic metabolic disorder in accordance with the literature. The analysis of a patient group with type-1 diabetes at the initiation of their insulin pump treatment ensures a unique option to draw conclusions on the consequences of poor glycemic control. The overall grade of AN was not high, as the mean AN score was 2.85, but the relationship was strong between the duration of diabetes and the severity of the autonomic dysfunction. In the presence of longer disease duration, a more severe AN was proven by the reflex tests. These data are in accordance with our previous findings that the variability of the glucose levels is in close relationship with the severity of AN. The detailed analysis of the possible correlation between each of the four reflex tests and the duration of diabetes revealed that the results of the most sensitive parasympathetic tests, the heart rate response to deep breathing are less physiologic in the presence of a longer metabolic disease. According to some

opinions, this test alone may be suitable for detecting the presence of autonomic neuropathy, while performing other procedures at the same time may support determining the severity. This observation leads to a conclusion that mainly parasympathetic impairment is expected in type 1 patients at the initiation of pump treatment. Moreover, this condition is frequently associated with a relative dominance of the sympathetic function resulting in increased cardiovascular risk for these patients. The data suggest that in this patient population, exposure to diabetes prior to pump application caused a moderate degree of AN, the first manifestation of which was impaired parasympathetic function. A significant short-term improvement was found in the overall cardiovascular autonomic function during the follow-up of the pump treatment. 2 months after the pump application the autonomic score became significantly lower. The analysis of the separate tests did not reveal a significant change in the results by the second month but 3 of the 4 tests reflected a tendency of an improving cardiovascular function. The significant reduction of the autonomic score might be a cumulative additive result of the partial improvement in the parasympathetic and sympathetic functions. The beneficial effect during such a short period of the intensified glycemic control on the cardiovascular autonomic function was not published earlier in type 1 diabetic patients. These data might support the hypothesis that the moderate impairment of autonomic regulation might be sensitive for the short-term changes of the metabolic conditions and the pathogenetic process is particularly reversable. The results of the follow-up after 6 years reflect the same severity of neuropathy as it was recorded at the baseline tests. This means that the autonomic function was preserved during a 6 year-long period with the most intensive insulin treatment. The degree of the glycemic control characterized by HbA1c didn't change by the second month but became significantly lower by the 6^{th} year, although the mean value didn't reach the glycemic target. The initial moderate tendency of improvement in the autonomic function seemed to be temporary by the 6^{th} year. Our trial suggests that the beneficial effect might begin as early as months after the intensive treatment and lasts up to 6 years. Our observations on type 1 diabetic patients draw the attention to the importance of the earliest stabilization of glycemic control as far as possible in order to prevent the progression of autonomic neuropathy.

6 Conclusions and new findings

1. The cardiovascular autonomic function is not altered in young women with PCOS besides the presence of putative risk factors.

2. A hypersensitive peripheral sensory condition is proven in the upper and lower extremities in PCOS patients. This sensory hyperfunction may be regarded as a very early manifestation of neuropathy.
3. Patients with poorly controlled type 1 diabetes has a moderately severe cardiovascular autonomic neuropathy at the start of their pump therapy when their metabolism required a change in therapy.
4. Duration of type 1 diabetes has been consistently associated with the severity of parasympathetic neuropathy as a manifestation of early autonomic nervous system damage.
5. During two-month pump treatment, cardiovascular autonomic function moderately improved after a relatively rapid correction of the metabolic state.
6. The severity of the cardiovascular autonomic neuropathy was preserved during a 6 year-long period with the most intensive insulin treatment in type 1 diabetes.

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