THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

ELECTRONEUROGRAPHIC EXAMINATIONS IN SOME DISEASES WITH THE INVOLVEMENT OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

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Supervisors: Péter Diószeghy MD, PhD, Dániel Bereczki MD, PhD, DHAS

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1. INTRODUCTION

1.1. THE ROLE OF ELECTRONEUROGRAPHY IN THE DIAGNOSIS OF THE PERIPHERAL NERVE DAMAGES

Electroneurography (ENG) is a clinical electrophysiological method for the evaluation of the type and severity of the peripheral motor and sensory nerve damages. The ENG is first of all used in the diagnosis of polyneuropathies, mononeuropathies, radicular lesions, tunnel syndromes and nerve dagames caused by trauma and compression. The nerves are stimulated with surface (rarely with needle) electrodes, and the answers are detected with surface electrodes fixed on the skin. The recording place of the motor potential is above the target muscle, and of the sensory potential is above the sensory nerve. The latency, amlitude, duration, area of the motor and sensory potentials and the motor and sensory nerve conduction velocities could be calculated. We can evaluate the severity and type (axonal, demyelinating, or mixed form) of the nerve damage comparing these parameters with the normal values.

1.2. THE TERMAL SENSITIVITY OF THE PERIPHERAL NERVES

It is well known that nerve conduction parameters (latency, amplitude, duration, area, conduction velocity) alter with the changing of temperature. This is not surprising, because many structures of the neuromuscular system, such as voltage-gated ion channels, or the acetylcholinesterase enzyme activity have temperature sensitivity. Each component shows a unique temperature profile, that's why the effects of cooling and heating can be complex.

1.3. THE INVOLVEMENT OF THE PERIPHERAL NERVES IN DIABETES MELLITUS

Diabetes affects 1-4% of the world population, and according to the statistics of the WHO the prevalence of the disease will be doubled from 1995 to 2010. The most common complications of the disease are polyneuropathy, macro-, and microangiopathy, retinopathy and nephropathy. The prevalence of polyneuropathy is approximately 50% in diabetic patients. It is generally agreed that the severity of the peripheral nerve damage is first of all under the influence of the duration of diabetes, and the poorly controlled blood glucose level. The most common symptoms of the diabetic polyneuropathy are paresthesias, pain, hyp/or anesthesia, decrease, or absence of the deep tendon reflexes, atrophy and paresis of the involved muscles. With the progression of the disease there could be other complications, as the „diabetic foot", which often leads to amputation. The „diabetic foot" develops on the basis of polyneuropathy, macro-, and microangiopathy, and this is the most common cause of death in type I diabetic patients. The cardio- and cerebrovascular diseases are the consequences of the micro- and macroangiopathy. Among these the myocardium infarction and the sroke have high priority, wich play important part in the diabetic mortality. The damage in the cerebrovascular reativity causes decreased cerebrovascular reserve capacity (CRC).

1.4. THE INVOLVEMENT OF THE PERIPHERAL NERVES IN MULTIPLE SCLEROSIS (MS)

The pathological characteristics of the disease is an autoimmune inflammatory and degenerative process resulting in demyelination in the central nervous system (CNS), that is followed by remyelination. This is clinically characterized by the relapsing-remitting course of the disease in most patients at the beginning of the disorder. Axonal damage also occurs, resulting in permanent and progressing neurological deficits. In the primary progressive

form of MS axonal injury could develop in the early phase. Although MS is basically the disorder of the CNS, the idea that the peripheral nerves might also be affected has been coined in 1903. Since then several observations in electrophysiological and neuropathological studies have suggested that the peripheral nervous system (PNS) might indeed be a target organ in MS. The most common findings were prolonged distal latency, decreased amplitude and conduction velocity, increased jitter, and reduction in myelin thickness.

The symptoms of MS patients may worsen during elevation of temperature (Uhthoff's phenomenon), because the conduction becomes disturbed in the partially demyelinated fibers in the CNS. The effect of changing temperature (i.e. cooling and heating) on the electrophysiological characteristics of the peripheral nerves of MS patients in a follow-up study has never been published to our knowledge.

1.5. THE INVOLVEMENT OF THE PERIPHERAL NERVES IN MITOCHONDRIAL DISORDERS

The mitochondriopathies are not rare, and thanks for the modern diagnostic procedures more and more disorders prove to be in this group. The most well-known syndromes are the mitochondrial myopathy (MM); the chronic progressive external ophthalmoplegia (CPEO); the mitochondrial myopathy, epilepsy, ragged red fibre syndrome (MERRF) and the mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS).

The most common symptoms in the mitochondrial disorders are myopathy, neuropathy, encephalopathy, pigmented degeneration of the retina, hypacusis, epileptic seizures and cardial problems. The diagnosis is complex. The lactic acid level of blood is elevated. Electromyography (EMG) reveales predominantly myogen, but sometimes neurogen, or mixed type lesion of the skeletal muscles, the ENG showes mild, primarily axonal and sensory type of polyneuropathy. Light microscopic analysis showes "ragged red" fibers and

cytochrom oxidase C (COX) negative fibers in the skeletal muscles. Pathological mitochondria were detected by electronmicroscopic examinations. The morphometrical evaluation of the sural nerves verified predominantly axonal type of polyneuropathy in mitochondrial disorders. On one hand the pathological amount and structure of mitochondria in the endothel of vasa nervorum, in the perivascular smooth muscle cells and in the pericytes could cause microangiopathy and afterwards polyneuropathy. On the other hand the damage of the peripheral nerves can be provoked by systemic mitochondrial cytopathy.

2. OBJECTIVES

In our complex electrophysiological study the first aim was the construction of a reliable electroneurographical score wich reflects objectively the clinical severity of polyneuropathy.

In the diabetic group we wanted to answer the folowing questions:

- Can our electroneurographical score reflect objectively the clinical severity of diabetic polyneuropathy?
- Does the thermal sensitivity of nerve conduction parameters change with the severity of neuropathy in the groups of various severity of polyneuropathy categorized by the electrophysiological score compared with healty controls?
- Is there correlation between the severity of peripheral neuropathy and the impairment of cerebrovascular reserve capacity (CRC) in diabetic patients evaluated by nerve conduction studies and transcranial Doppler?

We hypothesized that the peripheral nervous system is also affected in multiple sclerosis and we also assumed that these changes might be more prominent at lower or at higher temperatures and the nerve conduction parameters would worsen in parallel with the progression of the disease.

In the MS group we wanted to answer the folowing questions:

- Do the nerve conduction parameters differ between MS patients and healthy subjects at room temperature?
- Does the temperature have similar, or different effect on the peripheral nerve conduction parameters in MS patients compared with healthy subjects?
- How do these parameters change during a three-year follow up?
- To evaluate whether the clinical progression of the disease can be reflected by changes in electrophysiological parameters.

In mitochondriopathies we wanted to answer the folowing questions:

- Can we detect any abnormalities in the function of the peripherial nerves in mitochondrial disorders?
- If we could detect: what kind of type and severity are these abnormalities?
- Are there any differences in the nerve functions in the three examined groups (MM, CPEO, MELAS)?

3. PATIENTS AND METHODS

3.1. THE ELECTRONEUROGRACHICAL SCORE (ENG SCORE)

3.1.1. The structure of the ENG score

We wanted to quantitate the severity of polyneuropathy with measuring the electroneurographical parameters of several motor and sensory nerves. The motor and the sensory parameters of the median and ulnar nerves were recorded on one side, and those of the peroneal and sural nerves on both sides. Because we measured the electophysiological parameters in two segments of the motor fibers of the ulnar and peroneal nerves and in one segment of the motor fibers of the median nerve and of the sensory fibers of the median, ulnar and sural nerves, we had 11 nerve segments altogether. We measured the

conduction velocities and the amplitudes of the motor and the sensory potentials and the distal latency of the peroneal nerves. The findings were compared with generally accepted normal values. The severity of polyneuropathy was scored by a numerical value ranging from 0 to 79, which was calculated by summing the motor and sensory dysfunction indicator points of the items. If the conduction velocities, amplitudes, distal latencies were normal on all nerves, then the score was 0, if there wasn‘t any motor and sensory answer on any of the nerves, then the maximal amount of points (79 points) were given. On the basis of the score we divided the electoneurographic grade of polyneuropathies into three groups: between 1 and 26 points the polyneuropathy is mild, between 27 and 52 points moderate and between 53 and 79 severe.

3.1.2. The validation of the electroneurographical score

We wanted to evaluate if the ENG score reflected genuinely the clinical severity of polyneuropathy. The clinical severity of neuropathy was assessed by the presence or absence of sensory signs, reflex alterations, and paresis/atrophy. Depending on the clinical findings, a score of either 0 or 1 was given for each of the 3 items, therefore a total clinical score was 0 if neither sensory disturbance, nor hyporeflexia or paresis/atrophy was observed, and a clinical score of 3 meant that the patient had sensory disturbance as well as hypo-/areflexia and paresis/atrophy. The clinical and electroneurographic scorings were done by two independent observers. To confirm the validity of scoring, we compared the electrophysiological scores in 5 groups based on clinical signs of neuropathy: healthy controls, diabetics with no signs of polyneuropathy, diabetics with mild, moderate and severe clinical signs. We also used the Spearman rank order test to correlate the clinical score to the electrophysiological score.

3.2. THE DIABETIC PATIENTS

3.2.1. The subjects in the temperature dependence study of the nerve conduction parameters

Type I and II diabetic patients of the Diabetes Outpatient Service of the 1st Department of Internal Medicine, University of Debrecen, and diabetic patients treated at the Department of Neurology, University of Debrecen were enrolled in the study. After explaining the study procedures all of them agreed to participate. Those who had any other diseases known to be associated with peripheral neuropathy were excluded from the study. During the study period 77 diabetic patients had an electrodiagnostic study. By the electrophysiological score the severity of polyneuropathy was categorized to mild, moderate and severe. Into the thermal sensitivity study the inclusion of the diabetic patients was consecutive. When we reached 10 patients in each group we stopped the inclusion to that group. Therefore in the thermal sensitivity study 10 patients had mild, 10 had moderate and another 10 had severe polyneuropathy. Healthy employees of the Department of Neurology of the University of Debrecen and their healthy relatives were analyzed as controls.

3.2.2. Patients in the correlation study between the severity of diabetic polyneuropathy and the cerebrovascular reserv capacity (CRC)

Type II diabetes mellitus patients of the Diabetes Outpatient Clinic of the 2nd Department of Internal Medicine University of Debrecen, were enrolled in the study. The inclusion criteria were: age >18 years, no previous cerebrovascular disease in the history. Patients were evaluated by duplex scanning and those with hemodynamically significant stenosis of the carotid and vertebral arteries were excluded from this study.

3.3. PATIENTS WITH MULTIPLE SCLEROSIS

All patients with relapsing-remitting course of MS returning for a checkup examination to the Neuroimmunological Outpatient Service of the Department of Neurology, University of Debrecen, where the diagnosis was determined on the basis of the criteria of Poser and McDonald were asked to participate in the study. At the time of the basic and follow-up examinations all patients were in stable condition. 11 of our 13 MS patients were treated with immunomodulatory drugs. Those who had any diseases known to be associated with peripheral neuropathy were excluded from the study. Healthy employees of the Department of Neurology were analyzed as controls.

3.4. PATIENTS WITH MITOCHONDRIAL DISORDERS

Twelve patients with mitochondriopathies diagnosed in the Department of Neurology, University of Debrecen were enrolled in the study. The diagnosis was based on clinical, histological, biochemical and sometimes genetic investigations. Six patients with MM, 4 with CPEO, and 2 with MELAS were examined with ENG. Those who had any diseases known to be associated with peripheral neuropathy (e.g. alcohol abuse, diabetes mellitus) were excluded from the study.

The participants signed a consent form and the study was approved by the Ethics Committee of the University of Debrecen.

3.5. THE EXAMINATION OF TEMPERATURE DEPENDENCE OF THE NERVE CONDUCTION PARAMETERS

3.5.1. Motor nerve studies

The right median nerve was stimulated with a bipolar surface electrode using square wave pulses of 0.1 msec duration. The stimulus was delivered at the wrist (with a constant distance of 8 cm proximal to the recording electrode) and at the elbow. A surface recording disc electrode was attached over the endplate region of the abductor pollicis brevis muscle. The reference electrode was placed on the distal interphalangeal joint of the thumb.

The following motor nerve parameters were measured at all temperatures: latency, amplitude, duration, area of the compound motor action potential (CMAP) and the motor conduction velocity (MCV). The latency, amplitude, duration and area could be distal and proximal depending on whether the stimulation site was at the wrist, or at the elbow.

3.5.2. Sensory nerve studies

The right median nerve was stimulated orthodromically with a bipolar surface electrode using square wave pulses of 0.1 msec duration on the radial side of the third digit. The sensory potentials were recorded with bipolar surface felt pad electrodes at the same points as used for motor nerve stimulation at the wrist approximately 14-15 cm from the stimulation point.

The following sensory nerve parameters were measured at all temperatures: latency, amplitude, duration, area and the sensory conduction velocity (SCV) wich was calculated for the nerve segment between the third digit and the wrist.

3.5.3. The method of measurements at different temperatures

After obtaining the first motor and sensory records at room temperature the forearm including the elbow was cooled in a thermostated water bath at 20ºC for 10 minutes. The upper extremity was then lifted from the bath and dried, the electrodes were reapplied over the marked points and the recording was performed again. Then the arm was immersed in the water again and the warming started. The water temperature was changed stepwise to 25, 30, 35

and 40ºC. At each temperature the arm was in the water bath for 10 minutes before the nerve conduction examinations. The skin temperature was measured just before the stimulation at the site of the recording and stimulating electrodes at the wrist with a digital infrared thermometer (TFI 497 IR thermometer, Ebro Electronic, Ingolstadt). The thermometer had an accuracy of 0.5ºC. Its response delay was ≤1 sec.

3.6. THE MEASUREMENT OF THE CEREBROVASCULAR RESERVE CAPACITY (CRC)

Patients were assessed in the supine position by the 2 MHz probe of the DWL-7 transcranial Doppler (DWL, Sipplingen, Germany). The middle cerebral artery was insonated through the temporal window at 50 mm depth. Peak, mean and diastolic blood flow velocities, as well as pulsatility indices were recorded after at least a 5 minutes rest at supine position. After assessing resting cerebral blood flow velocities, 1000 mg acetazolamide was injected slowly intravenously. Blood flow velocity measurements were repeated at 5,10,15 and 20 minutes after injection. CRC was defined as the largest percent increase of the middle cerebral artery mean blood flow velocity and was calculated as follows:

$$CRC=\frac{(MCAV_{max}-MCAV_{rest})\ x\ 100}{MCAV_{rest}}$$

Where $MCAV_{max}$ is the maximal increase of the middle cerebral artery mean blood flow velocity, $MCAV_{rest}$ is the resting blood flow velocity of the middle cerebral artery, and CRC indicates cerebrovascular reserve capacity in %.

3.7. LABORATORY TESTS IN THE CORRELATION STUDY BETWEEN THE SEVERITY OF DIABETIC POLYNEUROPATHY AND THE CEREBROVASCULAR RESERV CAPACITY (CRC)

Before injecting acetazolamide, blood was taken for the following laboratory investigations: blood glucose, glycosilated haemoglobin, actual insulin concentration, VIII. factor-related antigen, alpha-2 macroglobuline. Urine microalbumin concentration was measured after a timed collection lasting for 24 hours.

3.8. STATISTICAL METHODS

3.8.1. *Temperature dependence studies of the nerve conduction parameters* in diabetic and MS patients

Normality of the continuous variables was checked by the Shapiro-Wilk test. Because in diabetic patients we detected normal distribution in almost all the measured parameters, we used the ANOVA test. Based on the distribution of the variables, the paired and unpaired t-tests, or the Mann-Whitney and the Wilcoxon matched pairs tests were used in MS patients. Repeated measure ANOVA was used to test if the changes in nerve conduction parameters run parallel in patients and controls while heating the arm from 20 to 40 ^{o}C. Changes in electrophysiological parameters normalized to unit change in temperature were also compared.

3.8.2. Diabetic polyneuropathy and cerebrovascular reserve capacity

Means±standard deviations are reported. Normality of the parameters was checked by the Saphiro-Wilk test. Multiple regression was used for the assessment of the correlation between the neuropathic score and cerebrovascular reserve capacity, age of the patients, duration of diabetes, actual glucose, insulin concentration, alpha2-macroglobulin and VIII-factor-related antigen. ANOVA was used to compare parameters between subgroups.

Statistica for Windows 5.5 and 6.1 (StatSoft, Tulsa, USA) were used for data analysis. Statistical significance was assumed if $p<0.05$.

4. RESULTS

4.1. VALIDATION OF THE ELECTRONEUROGRAPHICAL SCORE

To validate the electrophysiological score, first we tested whether the score differs in subjects with various clinical severity of diabetic neuropathy. We have found, that the the electrophysiological score is higher in patients with more severe signs of neuropathy ($P < 0.001$, Kruskal-Wallis ANOVA). The Spearman correlation also reflects that more severe clinical neuropathy is associated with higher electrophysiological score (Spearman $R = 0.82$, $p<0.001$).

4.2. TEMPERATURE DEPENDENCE OF THE NERVE CONDUCTION PARAMTERS

4.2.1. Temperature dependence of the nerve conduction parameters in diabetes mellitus

4.2.1.1. Electrophysiological parameters of the median nerve at room temperature

First we applied between-group comparison of the electrodiagnostic tests performed on the median nerve at room temperature. We studied if there were any statistical significant differences among the control and the three diabetic groups. There were statistically significant differences in practically all the measured parameters. The CMAP distal ($p=0.063$) and proximal ($p=0.059$) durations and the proximal areas ($p=0.082$) were on the margin of statistical

significance. The distal and proximal motor latencies, the sensory latencies and the duration of the sensory potentials were longer in diabetic patients compared to the controls, and the increases in these values were more prominent in patients with more severe polyneuropathy. The distal and proximal amplitude, duration, and area of the compound motor action potentials, the motor conduction velocities and the amplitude and area of the sensory potentials and the sensory conduction velocities were smaller in diabetic patients compared to the controls, and the decrease in these values were more prominent with more severe neuropathy.

4.2.1.2. Changes in median nerve conduction parameters related to changes in temperature

Nerve conduction parameters were measured on the right median nerve at 5 ^{o}C increments, between 20 – 40 ^{o}C. The distal and proximal motor and sensory latencies, the amplitude, duration, and area of the motor and sensory potentials increased, the motor and sensory conduction velocities decreased with lower temperature levels both in the control and diabetic groups. Repeated measure analysis of variance was used to compare the reaction of various electrophysiological parameters during the course of temperature change in the three diabetic groups and in controls. Here the temperature of the thermostated water bath was used as a measure of environmental temperature. In this analysis the main effect of the group, the main effect of the environmental temperature, and their interaction were considered. We found significant group main effect and temperature main effect in all the measured parameters. There was significant group-temperature interaction in the distal and proximal motor areas ($p<0.001$ for both), in the sensory areas ($p<0.001$) and in the sensory conduction velocities ($p<0.001$).

4.2.1.3. Temperature-normalized changes in median nerve conduction parameters

When analyzing changes in nerve conduction parameters normalized to 1 ^{o}C change in skin temperature in the 40 - 20 ^{o}C range, we performed between-group comparisons. We found statistical significant differences among groups in the same parameters as above in the group-temperature interactions analysis: in the distal and proximal motor areas ($p<0.001$ for both), in the sensory areas ($p=0.002$) and in the sensory conduction velocities ($p<0.001$). The more severe was the polyneuropathy, the less difference appeared during 1 C° temperature changing.

4.2.2. Temperature dependence of the nerve conduction parameters in multiple sclerosis

4.2.2.1. Basic electrophysiological parameters and comparison of the baseline to 3 years values at room temperature

At the baseline measurements the mean EDSS score in the patients was 4.3±2.3 and statistically significant disease progression was detected by 3 years (4.8±2.3, $p=0.040$) with the Wilcoxon matched pairs test.

(a.) First we performed between-group comparisons both at baseline and at 3 years, then (b.) we compared the baseline values to the corresponding follow up data within groups.

a. There were nearly significant differences between the groups in a few nerve conduction parameters: at the initial examination the distal (3.45±0.36 and 3.82±0.59 msec, $p=0.058$) and the proximal (7.06±0.68 and 7.61±0.91 msec, $p=0.09$) motor latencies tended to be longer in the MS group compared to the controls and 3 years later the same was seen in the proximal motor latencies (7.2±0.4 and 7.77±0.92 msec, $p=0.06$).

b. There was no statistically significant change in any of the motor and sensory parameters in the control group between the 2 measurements. In contrast with the findings in the controls, in MS patients the CMAP distal area (29.13±7.90 and 33.0±8.96 mVmsec, p=0.026), the CMAP proximal duration (7.66±1.91 and 9.11±2.47 msec, p=0.023) and the CMAP proximal area (26.88±8.99 and 33.28±9.65 mVmsec, p=0.007) significantly increased during 3 years, the CMAP distal duration also tended to be higher (8.22±1.88 and 9.36±2.53 msec, p=0.074). There were no statistically significant changes in the course of 3 years in the sensory parameters of the MS group.

4.2.2.2. Changes in nerve conduction parameters related to changes in temperature

a. There were nearly significant differences in nerve conduction parameters normalized to unit change in temperature between healthy controls and MS patients: both at the initial and the 3 years later repeated measurements for tempeature normalized CMAP distal and proximal duration and area were almost significantly higher ($p<0.1$) in MS patients compared with the healthy controls.

b. There was no statistically significant change in any of the conduction parameters of the controls at 3 years compared to the baseline at any temperatures. In MS patients, significant or close to significant increase of distal and proximal CMAP durations and areas were found at almost all temperature levels at 3 years compared to baseline. There were no statistically significant changes in the sensory parameters in the MS group at any temperature at 3 years compared to baseline.

Nerve conduction parameters normalized to unit change in temperature did not differ in controls at 3 years compared to baseline. In the MS group both distal motor latencies and sensory latencies normalized to unit change in temperature decreased significantly in 3 years compared to the initial measurement

(0.19±0.04 and 0.15±0.04 msec/^{o}C, p=0.006; and 0.17±0.04 and 0.14±0.05 msec/^{o}C, p=0.045).

4.2.2.3. Effect of environmental temperature on electrophysiological parameters – comparison of changes between MS patients and controls

Repeated measure analysis of variance was used to compare the effect of temperature on various electrophysiological parameters in MS patients and controls. In the analysis the main effect of the group (i.e. control or MS), the main effect of the environmental temperature, and their interaction were considered. The proximal and the distal motor latencies were that parameters, where both significant main effects and significant group with temperature interactions were found**,** so: in these parameters after pooling the measurements at different temperatures in the MS group and the controls differed from each other, and the course of the proximal and the distal motor latencies did not run parallel in controls and patients as the temperature changed.

4.3. RELATIONSHIPS, WICH WERE EXAMINED DURING THE STUDY OF POSSIBLE CORRELATION BETWEEN THE DIABETIC POLYNEUROPATHY AND THE CEREBROVASCULAR RESERVE CAPACITY

4.3.1. Relationship between glycemic control and the assessed parameters

Based on glycosylated hemoglobin content, patients were divided into two groups: patients with previous good glycemic control (HbA1c ≤ 10%) and patients with recently poor glycemic control (HbA1c > 10%). Patients with inappropriate glycemic control had significantly higher concentrations of blood glucose, urine microalbumin and von Willebrand antigene (VIII-factor related antigene) concentrations. Cerebrovascular reserve capacity indicating cerebral microvascular function was the same in the 2 subgroups, whereas diabetic

neuropathy tended to be less severe in the better treated patients (13.0±8.3 vs. 20.6±10.2, p = 0.096).

4.3.2. Correlation between cerebrovascular reserve capacity and the assessed parameters

No relationship could be observed between cerebrovascular reserve capacity and age (p=0.13), duration of diabetes (p=0.12), actual blood glucose concentration (p=0.55), actual insulin concentration (p=0.79), HbA1c (p=0.62), microalbuminuria (p=0.54), alpha-2 macroglobuline (p=0.89) and VIII-factor-related antigene level (p=0.52). However, when the patients were divided by the duration of diabetes into 2 subgroups (≤ 15 years or > 15 years) the subgroup with longer diabetes duration had significantly more severe impairment of CRC (35 ± 14% vs. 50±15%, p = 0.036, n=10 in both subgroups).

4.3.3. Relationship between severity of polyneuropathy and the other assessed parameters

Using linear regression analysis, no correlation was found between the severity-score of the electroneurographically-assessed diabetic neuropathy and any of the investigated parameters, except microalbuminuria. In the subgroup with longer (over 15 years) diabetes duration polyneuropathy as reflected by the neuropathic score tended to be more severe (19.1±10.5 vs. 11.5±6.7, p = 0.07). When we divided the patients into 2 subgroups by the severity of neuropathy (neuropathic score ≤ 15, or > 15, n = 10 in both subgroups), microalbuminuria was significantly higher in the subgroup with more severe polyneuropathy (28.8±11.7 vs. 17.2±9.4 mg/24 hours, p=0.02).

CRC however did not differ significantly between these subgroups (48±17% vs. 38±15%, p = 0.18), the mean value of CRC was actually higher in the subgroup with more severe polyneuropathy. We performed multiple variance

analyses to check whether the confounding effect of covariates like the presence of hypertension, the age of the patients or the duration of diabetes – all known to influence CRC - could have resulted in this finding. When the presence of hypertension was entered as a single covariate, or when age and diabetes duration were also entered as covariates, CRC was still found to be independent of the severity of peripheral neuropathy ($p = 0.41$, and $p = 0.24$, respectively). Thus, a statistically significant relationship could only be observed between the severity of diabetic polyneuropathy and nephropathy. In contrast, no relationship could be detected between severity of diabetic neuropathy and the impairment of cerebrovascular reserve capacity, suggesting that cerebral vasoreactivity is not (or not much) influenced by neurogenic factors in type II diabetes mellitus.

4.4. THE TYPE AND SEVERITY OF POLYNEUROPATHY IN MITOCHONDRIAL DISCORDERS

The ENG revealed axonal type of polyneuropathy in 9 of our patients, predominantly axonal type in 2 and a predominantly demyelinating impairment in 1 case . According to our ENG score the polyneuropathy was considered mild in all the 12 examined patients (everybody reached 1-15 points). These points were so small, that we divided our patients into 2 subgoups: above 7 points the nerve damage was mild, with 7 points, or below the polyneuropathy was very mild. In our observed patient groups with mitochondriopathies (MM, CPEO, MELAS), the proportion of the very mild and mild polyneuropathies was almost the same.

5. DISCUSSION

5.1. The electoneurographical score (ENG score)

We constructed a reliable ENG score - analyzing 11 motor and sensory nerve segments - wich reflects objectively the clinical severity of polyneuropathy. As recommended by England et al a simple clinical scoring was used to validate the electrophysiological score. The statistical analyses verified the validity of our electroneurographical score: patients with more severe clinical signs had significantly higher ENG score.

5.2. TEMPERATURE DEPENDENCE OF THE NERVE CONDUCTION PARAMETERS

5.2.1. Temperature dependence of the nerve conduction parameters in diabetes mellitus

In diabetic polyneuropathy the latency and the duration of the potentials increase, the amplitude, the area of the motor and sensory potentials, and the conduction velocities decrease compared with the healthy peripheral nerve functions. The increase in latency and the slowing in conduction velocity are due to the degeneration of the myelin sheath. The amplitude and the area of the potentials depend on the number of the functional axons. The decrement of the amplitude and the area are on the basis of the axonal injury and the temporal dispersion. The expansion in the duration of the potentials is attributed to the temporal dispersion, when the conduction velocities of the nerve fibers scatter in a pathological wide range. The area could be seen as an integral function, as this is the integral of amplitude and duration.

At room temperature we found almost the same alterations in the nerve conduction parameters, except the distal and proximal motor durations, where

the differences were not statistically significant. The most prominent differences were in the distal and proximal motor amplitudes, in the sensory amplitudes, and the motor and sensory conduction velocities of the median nerve.

Cooling caused slowing of conduction velocity, prolongation of latency and increase in amplitude, duration and area of the recorded motor and sensory potentials both in diabetic patients and controls. The change in temperature primarily affects the kinetics of sodium channels. At lower temperature their opening time is longer, consequently additional Na ions move into the cells, a larger depolarization occurs resulting in an increase of amplitude, duration, area and rise time of the action potential.

In the 40 – 20 ^{o}C range there were significant differences in the same electrophysiological parameters in group-temperature interactions and in the temperature-normalized values: in the distal and proximal motor areas, in the sensory areas and in the sensory conduction velocities. The differences per unit change in skin temperature in the distal and proximal motor areas, in the sensory areas, and in the sensory conduction velocities were the largest in healthy controls and the smallest in the severe polyneuropathic group. According to some studies the variation per unit change in skin temperature in the conduction velocity ($\Delta v/\Delta T$) is smaller when the conduction velocity at room temperature is decreased because of nerve damage. In our study we examined not only the median nerve conduction velocities, but also a lot of other parameters of the motor and sensory potentials.The smaller differences of the electrophysiological parameters per unit change in skin temperature are not due to the lower basic values. In our hypothesis this reflects that the temperature sensitivity of some nerve conduction parameters decreases with the severity of diabetic polyneuropathy. It is well known, that in diabetes the peripheral nerves have increased resistance to ischaemia because of a reduced energy requirement and an increased efficiency of anaerobic glycolysis. In

more severe diabetic polyneuropathy the ischaemic resistance is more prominent. This alteration could be parallel with the more prominent temperature resistance. We found that the proximal and distal motor areas and the sensory areas show this temperature resistance most sensitively. The area depends on two parameters: on the amplitude and on the duration of the potentials. So this parameter is much more stable and could reflect the axonal loss and the severity of the polyneuropathy more precisely.

5.2.2. Temperature dependence of the nerve conduction parameters in multiple sclerosis

The differences were found between the healthy controls and MS patients we can summarize in 5 main points:

a. Statistically near significant alterations were found in the peripheral nervous system of the MS patients at room temperature: the distal and proximal motor latency were increased at the initial examination (p=0.058, p=0.09), and three years later we found the same tendency in the proximal motor latency values (p=0.06).

b. No changes between the initial and the 3-year values were found in healthy controls in any parameters at room temperature and at the different temperature levels. The involvement of the PNS in MS was clear by the follow-up measurements in our study: whereas we have found statistically significant, or close to significant increase in MS patients in CMAP distal and proximal durations (p=0.074 and p=0.023), and in areas (p= 0.026 and p=0.007) at room temperature and at almost all temperature levels during the follow-up measurements.

c. When temperature-normalized changes in nerve conduction parameters were evaluated, we detected the same tendency for differences in the same parameters: the CMAP distal and proximal durations and areas in MS patients

increased compared with the controls both at the baseline measurements and 3 years later ($0.05 < p < 0.1$)

d. Nerve conduction parameters normalized to unit change in temperature did not differ in controls at 3 years compared to baseline. In the MS group both distal motor latencies and sensory latencies normalized to unit change in temperature decreased significantly in 3 years compared to the initial measurement. This could be a sign of decreased temperature sensitivity in MS.

e. Increasing the environmental temperature from 20 to 40 ^{o}C, we found significant mean group effect and group-temperature interaction in distal and proximal motor latencies. So these parameters significantly differed in patients and controls ($p=0.04$ and $p= 0.024$), and the changes in these parameters did not run parallel in patients and controls as the environmental temperature changed ($p=0.046$ and $p=0.0004$).

The pathological characteristics of MS are an autoimmune inflammatory and degenerative process in the central nervous system (CNS). Several reasons may result in peripheral nerve damage in MS patients. Common backgrounds were assumed between MS and acute demyelinating polyneuropathy and probably hereditary motor and sensory neuropathy (HMSN). Higher anti GM1 antibodies were found in MS as well as in multifocal motor neuropathy. Some treatments used for MS might also result in neuropathy. Eleven of our 13 MS patients were treated with immunomodulatory drugs. This can not be held responsible for our findings as peripheral nerve damage is not enlisted among the frequent adverse events of these medications.

The lack of prospective studies was declared as one of the reasons for controversies in the relationship between MS and peripheral nerve damage. In our prospective study we have found the above mentioned (a., b., c., d. e.) results.

On one hand these findings suggest that demyelination could develop also in the peripheral nerves in MS (the increased latency indicates the damage of the

myelin sheath). On the other hand this could reflect the alteration in the kinetics of sodium channels during the progress of the diseases. It may be assumed that in MS – opposite with the healthy controls -even at higher temperature the opening and closing processes of these ion channels could become slower. In contrast to the motor features, there were few and nonsignificant changes in sensory parameters.

In this study we found that there are detectable differences in some nerve conduction parameters between MS patients and controls. We also found a tendency, that some parameters may react differently to changes in temperature in MS patients than in controls. Finally our results revealed that in 3 years some nerve conduction parameters changed in MS patients but not in healthy controls. These results support the suggestion, that the peripheral nervous system is also affected by the disease. It is undecided yet, why the motor nerves are more affected in MS. Among other mechanisms, reorganization of the extracellular matrix proteins, the role of antibodies against chondroitin sulphatase, against myelin associated glycoprotein, and against gangliosides, have been described as common factors in multiple sclerosis and peripheral motor nerve damage.

5.3. THE RELATIONSHIP BETWEEN THE DIABETIC POLYNEUROPATHY AND THE CEREBROVASCULAR RESERVE CAPACITY

Diabetes is known to be associated with impaired blood flow responses in the periphery as well as in the central nervous system both in type I and type II diabetes mellitus. Further, it was reported that some but not all measures of peripheral neuropathy correlated with the impairment of vasomotion in diabetes. For these reasons we hypothesized that one of the causes for decreased cerebrovascular reactivity in diabetes might be the impairment of autonomic innervation of cerebral resistance vessels. As diabetes mellitus

causes neuropathy affecting motor, sensory and autonomic nerves as well, we expected that the impairment of CRC develops in parallel with peripheral neuropathy, and checked this hypothesis by quantitative methods, i.e. transcranial Doppler and nerve conduction studies. To our best knowledge, this is the first study that assessed this issue.

The electroneurography is the most precise measure of the severity of polyneuropathy, that's why we used electrophysiological score when we related the severity of polyneuropathy to the impairment of cerebrovascular reactivity.

Because polyneuropathy is one of the most common complications in diabetes and the cerebral arterial tone is under the influence of neuronal factors, we tried to clarify the role of the impared neurogen activity in the decreased cerebrovascular reserve capacity in diabetes with indirect methods. We couldn't verify our hypothesis: *we couldn't demonstrate significant correlation* between the severity of polyneuropathy and the degree of impairment of CRC.

We assume that our neuropathic score truly represents the severity of diabetic neuropathy, this indicates that the lacking correlation between the severity of neuropathy and the decreased CRC is presumably not due to a methodological artifact of the ENG score.

A more probable explanation for the lacking correlation between nerve conduction and vasoreactivity changes could be due to the differences between the regulation of blood flow at the periphery and in the brain. The arteriolar tone within the brain is basically adjusted by cerebral autoregulation and cerebral metabolic regulation. Although neuronal factors also have a role in the determination of the cerebral arteriolar tone, their influence is presumably modificativ. Thus, it is conceivable, that this altered neurogenic activity has only a minor if any role on cerebral vasoreactivity.

We concluded, that the decreased CRC in diabetes is much more the consequence of structural changes in the resistance vessels and metabolic causes, than the result of neurogenic factors.

5.4. ELECTRONEUROGRAPHICAL ALTERATIONS IN MITOCHONDRIAL DISORDERS

The ENG revealed predominantly axonal type of polyneuropathy in all of our patients, wich involved first of all the sensory fibers. According to our ENG score the polyneuropathy was considered mild in all the 12 examined patients. We divided our patients into 2 subgoups: patients with mild , and with very mild type of polyneuropathy. In our observed groups with mitochondriopathies (MM, CPEO, MELAS) the proportion of the very mild and mild polyneuropathies was almost the same.

The electrophysiological examinations play very important role in the diagnostic procedure of mitochondriopathies, but for the definite diagnosis the morphological (neuropathological) examinations are essential.

6. SUMMARY

We evaluated the involvement of the peripheral nerves in patients with **diabetes mellitus**, **multiple sclerosis** and **mitochondrial disorders** with electoneurography. The diabetic polyneuropathy predominantly affects the peripheral nervous system, whereas the MS is first of all the disease of the central nervous system.The mitochondriopathies are „borderline cases" in this point of view, affect both the central and the peripheral nervous system.

First we constructed and validated an electroneurographic (ENG) score. Then we examined the thermal sensitivity of the median nerve with electroneurography in diabetic and MS patients compared with healthy controls.

The possible correlation between the decreased cerebrovascular reserve capacity and the severity of polyneuropathy in diabetes mellitus was also evaluated.

Finally we analysed the degree of the peripheral nerve involvement in patients with mitochondriopathies.

- The statistical analyses verified the validity of our ENG score: patients with more severe clinical symptoms had significantly higher ENG score.
- We found that the thermal sensitivity of some of the nerve conduction parameters (first of all the areas of the motor and sensory potentials) decreased with the progression of diabetic polyneuropathy.
- We have found increased distal and proximal motor latencies during the first and also the follow-up measurements in MS patients compared with the healthy controls, which could be the sign of demyelinisation of the peripheral nerves in MS. There was also worsening in some of the nerve conduction parameters (in the distal and proximal durations and areas of the CMAP) of MS patients, but we couldn't find any statistically significant changes in healthy controls in a three year follow-up study. These findings support the involvement of the peripheral nervous system in multiple sclerosis.
- We didn't find correlation between the severity of polyneuropathy and the impairment of cerebrovascular reserve capacity (CRC) in diabetes. Decreased CRC in diabetic patients might be rather due to structural changes of resistance arteries or to metabolic than to neurogenic factors.
- The ENG results were abnormal in every patients suffering from mitochondriopathy. The electroneurography revealed predominantly axonal and sensory type of polyneuropathy. The severity of the nerve damage - based on our ENG score - was mild in every cases.

7. PUBLICATIONS

7.1. PUBLICATIONS RELATED TO THE RESULTS OF THE PRESENT THESIS

Hidasi E, Molnár M, Diószeghy P, Mechler F. Electroneurographic and electromyographic alterations in mitochondrial disorders. Ideggyógy Sz 2001; 54:165-171

Hidasi E, Káplár M, Diószeghy P, Bereczki D, Csiba L, Limburg M, Fülesdi B. No correlation between impairment of cerebrovascular reserve capacity and electrophysiologically assessed severity of neuropathy in noninsulin-dependent diabetes mellitus. J Diabetes Complicat 2002; 16:228-234 **IF:1,649**

Hidasi E, Mechler F, Bereczki D. Etiology, pathology and treatment of diabetic polyneuropathy. Metabolizmus 2006; 3:217-221

Hidasi E, Diószeghy P, Csépány T, Mechler F, Bereczki D. Changes in nerve conduction parameters with temperature in multiple sclerosis. A three year follow-up study. Accepted for publication (Med Hypotheses) **IF: 1,276**

Hidasi E, Diószeghy P, Káplár M, Mechler F, Bereczki D. Temperature sensitivity of some nerve conduction parameters decreases with the severity of diabetic polyneuropathy. Submitted for publication (Exp Clin Endocrinol Diabetes)

7.2. PUBLICATIONS UNRELATED TO THE PRESENT THESIS

Mechler F, Diószeghy P, Glaub D, **Hidasi E.** The effect of levodopa in Parkinson's disease. Ideggyógy Sz 1996; 49:175-180

Hidasi E, Boczán J, Mechler F. Electrophysiological examinations in patients with amyotrophic lateral sclerosis. Ideggyógy Sz 1999; 52:388-394

Diószeghy P, **Hidasi E,** Mechler F. Study of central motor functions using magnetic stimulation in Parkinson's disease. Electromyogr Clin Neurophysiol 1999; 39:101-105

Hidasi E, Soltész P. The effect of the plasma exchange and other immunmodulant therapies on the course of severe Guillain-Barre syndrome. Orvosi Hetilap 2001; 142:335-339

Amadio S, Leocani L, Barnes M, Whittiker J, Bihari K, Anda J, Bogucki A, Jaworska-Chrebelska T, Burguera JA, Solis , Csala B, Tasnádi E, Csiba L, **Hidasi E,** Szabó A, Diószeghy P, Jónás C, Ferreira J, Costa , Coelho , Hefter H, Wunderlich G, Frohlich R, Moll M, Heywood P, Filipovic , Hyman N, Nairne A, Burton E, Lee M, Kukumberg P, Richter D, Benetin J, Lainez JM, Pascual , Ponz , Moore P, Tyne H, Rogg D, Murri L, Ludice A, Calabrese R, Reichel G, Hoffmann M, Ruhling J, Stenner A, Sangla S, Trocello JM, Selmaj K, Bieniek M, Berkowicz T, Serrano RA, Montes , Tugnoli V, Eleopra R, Swiatkiewicz J, Wierzbicki T, Szczudlik A, Rudzinska M, Banach M, Krygowska-Wajs A, Veselá D, Kahancova E, Wender M, Tokarz-Kupczyk E, Wygladalska-Jernas H. Botulinum toxin type B vs. type A in toxin-naive

patients with cervical dystonia: randomized, double-blind, noninferiority trial. Mov Disord 2008; 23(4):510-517. **IF: 3,207**

7.3. ABSTRACTS

Hidasi E, Diószeghy P, Mechler F. Evaluation of the central motor conduction time. X. Forum of Young Neurologists 1995

Hidasi E, Diószeghy P, Mechler F. Changing in the silent period, in the motor latency and amplitude using magnetic stimulation technic in Parkinsonic patients treated with L-dopa/Carbidopa. XI. Forum of Young Neurologists 1996

Hidasi E, Diószeghy P, Fülesdi B, Mechler F. Electroneurographic examinations in insulin and non insulin dependent diabetes mellitus. Electroenceph Clin Neurophysiol 1996; Vol. 9, No. 4:103.

Molnár M, **Hidasi E**, Gulyás B, Valikovics A, Molnár S, Mechler F. Correlative multimodal functional assesment of mitochondrial disorders. A potential tool for prognosis. Muscle Nerve 1998; (Suppl.7); S176

Hidasi E, Diószeghy P, Mechler F. Electroneurographical examinations in insulin dependent and non insulin dependent diabetes mellitus. National Congress of Hungarian EEG Society and Clinical Neurophysiology 1998

Hidasi E, Csépány T, Boczán J, Mechler F. Multimodal evoked potential examinations in MS patients treated with immunomodulant therapy. National

Congress of Hungarian EEG Society and Clinical Neurophysiology 1999, Ideggyógy Sz 2000; 53:59

Hidasi E, Molnár M, Mechler F. X chromosomal inactivation and dystrophin expression in Duchenne muscular dystrophy carriers. XXXXIII. National Congress of Hungarian Neurologists and Psychiatrists 2000

Hidasi E. The electrodiagnostic procedures of the peripheral neuromuscular complications in diabetes. National Congress of Hungarian EEG Society and Clinical Neurophysiology 2001

Hidasi E, Bereczki D, Csépány T, Mechler F, Diószeghy P. Changes in motor and sensory nerve conduction parameters with temperature in normal subjects and patients with multiple sclerosis. 2002 EFNS Eur J Neurol 9 (Suppl. 2); 145

Hidasi E. Ergot and non- ergot dopamin agonists. National Congress of Hungarian Parkinson's Disease Society 2006