Thesis for the Doctor of Phylosophy (Ph.D.) degree

Investigations in cerebrovascular risk factor and parameters affecting the outcome of ischemic stroke

Dr. Kerényi Levente

Tutor: László Csiba M.D., Ph.D., D.Sci

Department of Neurology

University of Debrecen

Medical and Health Science Center

2005

1. Justification of the research subject

Cerebrovascular disorders are the third leading cause of death and disability in the industrialised countries. The prevalence of stroke is different between these countries. Although there is improvement in Hungary in the past two decades, it is still several times higher compared to the western countries.

As the efficacy of stroke treatment is low, the prevention (primary and secondary) and the assessement and treatment of risk factors are very important. Non-treatable risk factors are age, gender and stroke in the family. The most important treatable risk factors are hypertension, diabetes mellitus, smoking, heart disorders carotid stenosis and hyperlipidaemia.

If somebody has a stroke, besides secondary prevention, it is very important to pay attention to the factors that influence stroke outcome like treatment in stroke unit, early rehabilitation, stroke localisation, type and size, the severity of symptoms, blood pressure, serum glucose level, body temperature and haemorrhagic transformation.

In the past two decades the neurosonological examinations gained important role in stroke diagnostics. Using the duplex ultrasound the examination of the carotid while using transcranial Doppler the examination of the intracranial arteries became possible. The advantages of them is that they are simple, non invasive and can be performed at bedside. It is possible to examine the intracranial and carotid arteries in different stroke risk factors, in acute stroke and can be easily combined with other methods (PET, SPECT, pathologic).

2. Introduction, aims

2.1. Cerebrovascular reserve capacity in patients with hyperlipidemia

Hyperlipidaemia is an important risk factor of atherosclerosis and of myocardial infarct. As recent data are conflicting, it is not certain whether hyperlipidemia is an independent risk factor for cerebrovascular diseases. Hypercholesterolemia has been observed in approximately one third of stroke patients in epidemiological studies. The relative risk of stroke in hypercholesterolemic patients is 1.4 times, in familial hypercholesterolemic patients 20 times higher than in the healthy population.

In recent years different tests were used for the assessment of cerebral hemodynamics in stroke-prone diseases. Cerebrovascular reactivity and reserve capacity was measured after the administration of acetazolamide. Acetazolamide (AZT) is a reversible inhibitor of carbonic anhydrase. The drug causes vasodilation of the brain resistance vessels (arterioles). After the administration of AZT cerebral arterioles dilate and cerebrovascular resistance decreases, resulting in an increase of cerebral blood flow which can be measured accurately and non-invasively with TCD. As hypercholesterolemia is a treatable risk factor of stroke, the TCD-acetazolamide test would be a valuable method for the early recognition and therapy of patients whose cerebrovascular reserve capacity is altered.

The aim of the study was to compare cerebrovascular reactivity, and reserve capacity in controls and hyperlipidemic patients.

2.2. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery

Hyperlipidaemia is an important risk factor of atherosclerosis. In studies using serial duplex ultrasound total cholesterol, HDL/total cholesterol ratio, LDL-cholesterol and triglycerides were predictors of plaque progression. However others did not confirm the

significant role of lipids as independent risk factors for carotid artery stenosis and its progression.

Because of the conflicting results our aims were: 1) to assess the relationship of total serum cholesterol, triglyceride and the severity of stenosis in the internal carotid artery 2) to assess if total serum cholesterol and triglyceride levels are independent risk factors of internal carotid artery atherosclerosis.

2.3. Blood pressure in the first week of ischemic stroke: its role in predicting outcome

Stroke is the most important disease among the disabling neurological disorders. It is a great burden on the patients, family and society. Therefore parameters affecting the improvement of stroke patients are of great importance. Neurological outcome is influenced by the stroke subtype, the severity of symptoms at admission, localization and size. However, other modifiable factors also affect stroke outcome. These are body temperature, hyperglycemia, erythrocyte sedimentation rate, fever, platelet count, cardiac disorders and hypertension. The role and treatment of hypertension in acute stroke are matters of controversy. Blood pressure increases immediately after acute stroke and spontaneously resolves within 7 to 14 days. Increased mean blood pressure was reported to be associated with poor prognosis in patients with impaired consciousness after acute ischemic stroke. High initial blood pressure, beat-to-beat diastolic, mean arterial pressure and its variability predicted poor functional outcome after acute ischemic stroke. Recently, Bath et al based on available results concluded, that high blood pressure (>140/90 mmHg) is independently associated with poor outcome in acute stroke.

So the aim of this study was to assess the role of hypertension and other parameters that influence acute ischemic stroke outcome using our stroke database, comparing the survival group with the lethal outcome group.

2.4. Factors influencing hemorrhagic transformation in ischemic stroke

Hemorrhagic transformation is a frequent complication of embolic stroke and also of thrombolysis in acute ischemic stroke. While the majority of hemorrhagic transformation (HTr) is not associated with worsening of neurological symptoms, symptomatic intracranial hemorrhage in the ischemic area worsens the outcome. As HTr may worsen the outcome of acute ischemic stroke, it is important to assess all risk factors influencing the hemorrhagic complications. Previous studies have already investigated the effects of the following parameters: systolic and diastolic blood pressure, body temperature, early ischemic signs on CT, treatment with anticoagulants, mean infarct volume, plasma matrix metalloproteinase-9, age, congestive heart failure, pretreatment with aspirin, and serum glucose level.

Because the autopsy can provide the most reliable data about the frequency of HTr (many of them clinically symptom free), our aim was to assess risk factors of HTr restricting our analysis on autopsied patients with or without HTr.

2. 5. Evaluation of intracranial hemodynamics in severe ischemic stroke patients.

Autoregulation of blood flow denotes the intrinsic ability of an organ or a vascular bed to maintain a constant perfusion in the face of blood pressure changes. Most likely, the autoregulatory vessel caliber changes are mediated by an interplay between myogenic and metabolic mechanisms. Autoregulation is lost in acute ischemic stroke, leaving surviving brain tissue unprotected against the potentially harmful effect of blood pressure changes. In

acute ischemis stroke edema developes in the infarcted area, the water contet of the brain increases. The basic mechanism of autoregulation of CBF is controversial, and the role of edema has not been examined comparing *in vivo* and *post mortem* results.

The purpose of this study was to examine the relationship between asymmetry of cerebrovascular reserve capacity (aCRC) and water content of the brain tissue analyzed postmortem.

2. 6. Cerebral effects of a single dose of vinpocetine in chronic ischemic stroke patients

Vinpocetine, a vinca alkaloid, increases cerebral blood flow by decreasing blood and plasma viscosity, platelet aggregability, and intravascular coagulation, and increasing erythrocyte deformability. In addition, vinpocetine has a neuro-protective effect. The *in vitro* effects of vinpocetine have been studied in great detail however, no detailed study has been performed to quantify the *in vivo* effects of vinpocetine on the regional cerebral blood flow in humans both under physiological and pathological conditions, including stroke and post-stroke states. Using transcranial Doppler (TCD) sonography it is possible to measure the cerebral blood flow velocity, the cerebrovascular reserve capacity, and the pulsatility index (PI) within the supplying territories of the middle cerebral arteries (MCAs).

The aim of the study was to examine the changes of these parameters in chronic ischemic stroke patients after vinpocetine treatment.

3. Patients and methods

3.1. Cerebrovascular reserve capacity in patients with hyperlipidemia

Between 1994 and 1997 outpatients with type II.a and II.b hyperlipidemia were studied. They were recruited from the Lipid Disorders Outpatient Clinic, and the outpatient department and hospitalized patients of our clinic. Hyperlipidemia was known for at least one year. All subjects had to be normotensive and normoglycemic, without cerebrovascular disease and diabetes mellitus in the history. Duplex scans of the carotid arteries (Ultramark 4 Plus, Advanced Technology Laboratories, Bothell, USA) were performed in all patients and had to show no or only mild (less than 30 % reduction in diameter) stenosis of the internal carotid arteries. Significant stenoses of the middle cerebral arteries were excluded based on the resting cerebral blood flow velocity as described earlier. Parallel with hyperlipidemic patients healthy controls (nurses, medical students, doctors, relatives) were also assessed in the same fashion.

Arterial blood pressure and heart rate were continuously registered during investigations. Before administration of acetazolamide blood was taken for the following laboratory investigations: total cholesterol, triglyceride, LDL-cholesterol, HDL- cholesterol, hemoglobin (Hgb), hematocrit ratio (Htc), red blood cell and white blood cell counts and fibrinogen. The investigators who performed transcranial Doppler tests were unaware of these characteristics during the test.

Middle cerebral arteries (MCA) were insonated on both sides using EME TC-2 64-B (Eden Medizinische Elektronik, Überlingen, Germany) transcranial Doppler equipment. A hand-held 2 MHz probe was placed at the temporal bone. Mean blood flow velocity in the middle cerebral artery was measured after a stabilizing period at rest and 5, 10, 15 and 20 minutes after intravenous administration of 1000 mg acetazolamide (Diamox®, Lederle

Parenterals, Carolina, Puerto Rico, USA) at 50 mm depth. Cerebrovascular reactivity and reserve capacity were calculated both in controls and hyperlipidemic patients.

Cerebrovascular reactivity (CVR) was calculated at each time point as percent increase of mean blood flow velocity in the middle cerebral artery after administration of acetazolamide. The maximal percent increase in blood flow velocity after acetazolamide (cerebrovascular reserve capacity=CRC) was also calculated.

Statistical analysis

Means ± standard deviation or standard error of mean are reported for all values. Parameters with normal distribution were compared with the appropriate unpaired t-tests. Repeated measure analysis of variance was used to detect differences in mean MCA blood flow velocity and cerebrovascular reactivity after acetazolamide administration between the hyperlipidemic and the control groups. Linear regression and multivariate regression analysis were used to assess the correlation between resting MCA mean blood flow velocity, cerebrovascular reserve capacity and serum cholesterol, triglyceride, fibrinogene levels and hematocrit ratio. Statistica for Windows v. 5.1 (Statsoft Inc. Tulsa, USA) software was used for statistical analysis. A $p<0.05$ value was accepted as statistically significant.

3.2. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery

The Debrecen Stroke Database was used, where there are about 100 parameters in the following categories: anamnesis, risk factors, clinical symptoms on admission, admission laboratory, ultrasound, CT and/or MRI results, and diagnosis. Over 3500 consecutive subjects of the database (between January 1994 and December 2000) 1934 acute ischemic stroke patients had both carotid ultrasound and lipid (total serum cholesterol and triglyceride level from fasting blood samples drawn after admission) measurements. Of the 1934 patient, all

risk factors were known in 1583 cases, while data on smoking habits were missing in 332 cases. The atherosclerotic change of the internal carotid artery was assessed by B mode and duplex ultrasound (HP SONOS 2000, Hewlett Packard) with a 7.5 MHz linear transducer. The carotid atherosclerosis was given in percent stenosis and patients in the database were divided into four groups: Group 1 (normal), group 2 (<30% stenosis), group 3 (30-99% stenosis) and group 4 (occluded).

Statistical analysis

Normality of serum cholesterol and triglyceride values was checked by the Saphiro-Wilk test. Kruskal-Wallis ANOVA was used to compare cholesterol and triglyceride values across the 4 groups of patients. The Pearson chi-square test was used to check for associations between the severity of carotid artery disease and gender, hypertension, diabetes and smoking.

Ordinal logistic regression was used to analyze the effects of cholesterol and triglyceride on the severity of carotid artery disease. The Wald statistic and its level of significance is presented. The level of carotid artery disease (normal, ≤ 30% stenosis, 30-99% stenosis, occlusion) was the dependent variable. In addition to cholesterol and triglyceride age, gender, smoking and hypertensive status were entered in the model. As gender and smoking were not independent predictors (men were more frequent smokers, male: 38.9%, female: 18.4%), two further models were tested omitting either smoking status or gender from the model. Statistica for Windows (v.6) was used for data analysis. A value of $P<0.05$ was considered statistically significant.

3.3. Blood pressure in the first week of ischemic stroke: its role in predicting outcome

Using our stroke and neuropathological database, factors affecting the survival of 346 acute ischemic stroke patients between January 1993 and December 1996 were evaluated.

Patients with primary intracerebral hemorrhage, hemorrhagic transformation or subarachnoid hemorrhage on admission CT or during autopsy were excluded. Patient selection: in the first step all consecutive deceased patients were selected if their data were complete. Selection of the survivor group: survivor patients of the same time period of the Stroke Database were ranked in alphabetic order. In the next step a computer generated set of random numbers was assigned to this list. In the final step, the patients were ranked by the random numbers and the first 170 patients on the list formed the surviving group.

The patients admitted to our hospital with symptoms of acute ischemic stroke received the same therapeutic and diagnostic regimen (brain CT, ECG, laboratory tests, carotid ultrasound, cardiologic examination, TTE or TEE). *None* of the patients received *rt-PA* treatment. Patients with severe paresis or plegia received heparin s.c. in prophylactic dose to prevent deep venous thrombosis. Blood pressure (BP) measurements were performed five times a day. The systolic and diastolic blood pressure values from admission to the seventh day were included in the analysis. Blood pressure was measured always on the same arm, and in case of difference between the arms, the higher blood pressure value was used. After death all patients were autopsied and the brains were removed and stored in formalin. For macroscopic evaluation 5-10 mm frontal slices of the whole brain were prepared and evaluated by an independent neuropathologist. Using this method it was possible to identify hemorrhage, lacunar and territorial infarcts and hemorrhagic transformation of ischemic lesions. The clinical findings were compared with the pathological results. A possible or probable cardioembolic stroke was defined based on the criteria of Kittner et al.

Primary outcome variable was stroke survival, and candidate explanatory variables were: age, sex, risk factors in the anamnesis (hypertension, diabetes mellitus, alcohol consumption: no, irregular, regular), smoking, TIA, cardiac disorders (atrial fibrillation, myocardial infarct, cardiac failure, angina), admission laboratory test results (taken within one

our after admission: erythrocyte sedimentation rate, hemoglobin, hematocrit, white blood cell count, platelets, serum Na, K, urea, glucose, cholesterol, triglyceride), therapy (anticoagulant, anti platelet), blood pressure (systolic, diastolic, maximal systolic, maximal diastolic from admission to the seventh day) and autopsy details (embolic origin, infarct size and localization). The infarct size was calculated on the brain CT and on the *post mortem* brain slices, and dichotomized (small: $\leq$10 cm^3, large: >10 cm^3).

Statistical analysis

Most of the variables needed no modification, but the multitude of data points describing the tendency of blood pressure in time warranted the introduction of summary measures. Thus, linear regression lines were fitted on blood pressure readings against time. This was done separately for systolic and diastolic values for each patient. As a result, information on blood pressure tendency from up to eight data points (first measured blood pressure of the day from days 0 to 7) was summarized in just two: first reading at admission (mmHg) and slope (mmHg/day) of the resulting regression line.

Separate simple Cox regression models were fitted using all available variables to find out whether they significantly influenced survival. All simple models were accompanied by an extended one containing the squared term of the explanatory variable if it was continuous. When this meant improvement of the model, these results were used and the value of the independent variable associated with the minimum or maximum of the hazard was also calculated. Two multiple models (systolic and diastolic), each based on the relevant four blood pressure variables (slope, slope squared, first reading, first reading squared), were fitted to evaluate the effect of blood pressure tendency on survival. To investigate the factors influencing survival in complexity, we fitted multiple Cox models including blood pressure variables as well as previously mentioned factors so as to estimate adjusted coefficients. Age, sex and blood pressure (slope and slope squared) were regarded as a priori adjustment

variables. The proportional hazards assumption was checked using a test based on Schoenfeld residuals. Statistical analysis was performed using Stata 8.2 (Stata Corporation) software.

3.4. Factors influencing hemorrhagic transformation in ischemic stroke

Using the database of our neuropathological laboratory, 245 consecutive acute ischemic hemispheric stroke patients (confirmed by CT at admission) between January 1993 and December 1996 were analyzed. Patients with primary intracerebral hemorrhage or subarachnoid hemorrhage on admission CT were excluded.

The patients admitted to our hospital with symptoms of acute ischemic stroke received the same therapeutic and diagnostic regimen (brain CT, ECG, laboratory tests, carotid ultrasound, cardiologic examination, TTE or TEE). None of the patients received rt-PA treatment. Patients with severe paresis or plegia received heparin s.c. in prophylactic dose to prevent deep venous thrombosis. Blood pressure (BP) measurements were performed five times a day. The maximal systolic and diastolic blood pressure values at admission, on the first, third and seventh day were included in the analysis. Blood pressure was measured always on the same arm, and in case of difference between the arms, the higher blood pressure value was used. After death all patients were autopsied and the brains were removed and stored in formalin. For macroscopic evaluation 5-10 mm frontal slices of the whole brain were prepared and evaluated by an independent neuropathologist. If necessary, histological staining was performed. Using this method it was possible to identify hemorrhage, lacunar and territorial infarcts and hemorrhagic transformation of ischemic lesions. The clinical findings were compared with the pathological results. A possible or probable cardioembolic stroke was defined based on the criteria of Kittner *et al*. To increase the reliability of our evaluation, chronic hypertension was defined if left ventricle hypertrophy could be found at autopsy. Baseline variable was stroke type, and candidate variables were: age, sex, risk factors

in the anamnesis: hypertension, diabetes mellitus, alcohol consumption (no, irregular, regular), smoking, TIA, cardiac abnormalities like atrial fibrillation, myocardial infarct, cardiomyopathy, angina, admission laboratory test results: erythrocyte sedimentation rate, hemoglobin, haematocrit, white blood cell count, platelets, serum Na, K, urea, glucose, cholesterol, triglyceride, therapy: anticoagulant, anti platelet, blood pressure: systolic, diastolic, maximal systolic, maximal diastolic at admission, on the first, third and seventh days, and autopsy details: embolic origin (cardiac), infarct size. The infarct size was calculated on the *post mortem* brain slices and dichotomized (small: $\leq$10 cm^3, large: >10 cm^3).

Statistical analysis

An exploratory logistic regression procedure was carried out to find the best multiple model identifying the factors associated with hemorrhagic transformation and estimating the effects thereof, expressed as odds ratios. This was started with simple models for each explanatory variable, where continuous variables were used in linear, logarithmically transformed, and categorized form. Blood pressure information was summarized in each component as the slope and intercept of a regression line fitted on blood pressure values versus time (days). The slope and the intercept values were used in the analysis. Multiple modeling was initially based on variables doubling the odds and/or significant ($p<0.05$) effects in the simple models, with age and sex as a priori confounders to control for. Other variables were added one by one, and were kept in the model if they improved it either by having significant effects on the outcome or by being active adjustment factors (either changed the significance or the odd ratio of other factors). Odds ratio estimates in the multiple model are corrected for all other explanatory variables. This means that the contrast or change in question entails the indicated odds ratio if all other factors are kept constant (i.e., in the comparison of people or groups of people who are different in the given contrast but not in anything else). Finally, interactions in the final model between age, sex, and other variables

were investigated, and then all possible interactions between variables not showing interactions with age or sex were checked. The fit of the final model was assessed using a Hosmer-Lemeshow goodness-of-fit chi-square test. Statistical analysis was performed by Stata 8.2 (Stata Corporation) software.

3.5. Evaluation of intracranial hemodynamics in severe ischemic stroke patients.

Twenty-four severe stroke patients who died within 72h (lethal outcome group) were studied with unilateral territorial infarct in the middle cerebral artery (MCA) territory. Neurogical and CT examinations were performed. The patients had no signs of increased intracranial pressure. The control group consisted of 24 age-matched healthy volunteers. Extracranial and intracranial supra-aortic vessels were investigated by duplex sonography (Ultramark 4 Plus, Advanced Technology Laboratories, Bothell, USA) and transcranial Doppler (TCD, EME TC2 64B, EME Überlingen, Germany). Cerebrovascular reactivity was measured using the acetazolamide-TDC test. After recording baseline blood flow velocities 1g acetazolamide was slowly injected intravenously. Values of capillary pH, pO_2, and pCO_2 were determined before and 20 min after the injection in each patient. Heart rate, blood pressure and mean velocity in the MCA (mFV) on both sides were recorded before and at 5, 10, 15 and 20 min after acetazolamide. The maximal percent increase in blood flow velocity after acetazolamide (cerebrovascular reserve capacity=CRC) was calculated. The asymmetry of CRC was calculated as affected/nonaffected side. In all patients who had died, autopsy was performed within 18h after death. Immediately after removal of the brain from the cranial cavity 8 tissue samples were taken out from the right and the left cerebral cortical area supplied by the MCA. The brain tissue samles were placed into preweighted vials and dried for 24h at 100°C. The perecent water content of the samples were calculated. The mean values of the eight samples were calculated for each hemisphere and correlated with the aCRC.

Statistical analysis

Velocity of blood flow between the two sides in the same subjects, as well as blood pH and blood gas data before and after acetazolamide administration were compared by the paired t test. Correlations between variables were analyzed using the Pearson correlation model and linear regression analysis. The significance of the correlation coefficients was evaluated using the Fisher z test. Bonferroni-corrections were applied for multiple comparisons.

3. 6. Cerebral effects of a single dose of vinpocetine in chronic ischemic stroke patients

Twelve chronic ischemic stroke patients participated in the study. The patients (8 men and 4 women; age 55-70 years; 62.4 ± 4.9 years, mean ± standard deviation) had ischemic stroke 13.4 ± 11.9 months (range, 5.5-41 months) prior to the current investigation. Five patients had a unilateral infarction in the region of the MCA, whereas in 7 patients the infarction was bilateral in the territories of the MCAs, with preponderance on one side. The infarcts were confirmed by two independent physicians using T2-weighted magnetic resonance imaging (MRI). TCD monitoring before and after vinpocetine treatment was performed with a TCD-EME-62-2B instrument (Eden Mediziniche Elektronik, Überlingen) using a 2-MHz frequency probe. The measurement depth was 55 mm from the probe. The parameters measured were the MCA mean blood flow velocity (MCAV; in centimeter per second), (2) percent velocity changes after treatment ([$MCAV_{after\ vinpocetine}$ − $MCAV_{before\ vinpocetine}$] / $MCAV_{before\ vinpocetine}$ x100), (3) velocity ratios between the symptomatic and contralateral sides, (4) pulsatility index (PI = [systolic peak velocity – diastolic peak velocity] / time-averged velocity), (5) percent changes of PI, and (6) PI ratios between the symptomatic and contralateral sides. Paired t-test was used for statistical analysis.

4. Results

4.1. Cerebrovascular reserve capacity in patients with hyperlipidemia

34 hyperlipidemic patients (12 male and 22 female, mean age: 45.55±11.51 years) and 21 controls (7 male and 14 female mean age: 42.52±15.37 years) were examined.

Total cholesterol, LDL-cholesterol and triglyceride levels were significantly higher in hyperlipidemic patients. There were no significant differences in age, arterial blood pressure, hemoglobin, red and white blood cell counts, platelet count and fibrinogen level between the hyperlipidemic patients and controls. Although hematocrit values were within normal range both in controls and hyperlipidemic patients, there was a statistically significant difference ($p=0.03$) between the groups.

There was a significant time main effect both in controls and hyperlipidemic patients, suggesting a significant increase of mean blood flow velocities after administration of AZT. In contrast, no group main effect could be detected, i.e. mean blood flow velocity in the middle cerebral artery at rest and after administration of acetazolamide was similar in controls and in hyperlipidemic patients.

No significant changes could be observed between controls and patients in cerebrovascular reactivity. Thus, percent increase of the middle cerebral artery mean blood flow velocity after AZT did not differ in controls and patients.

Maximal vasodilatory ability of the resistance arterioles was similar in healthy controls and hyperlipidemic patients.

No correlation was found between different laboratory parameters and resting cerebral blood flow velocity and cerebrovascular reserve capacity.

4.2. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery

The average age was 66.9±12.8 years (14-98 years, over 99% of the patients were between 35 and 94 years). Males (n=1068) were younger than females (n=866) (65.6±11.8 and 68.4±12.8 years, respectively, $p < 0.0001$). There was no significant gender difference in blood pressure, serum glucose level and stroke severity measured by the Mathew scale. The serum cholesterol level was higher in females than in males (6.3±1.4 and 5.8±1.3 mmol/L respectively, $p<0.001$), while serum triglyceride level was higher in men (1.75±1.14 and 1.65±1.13 mmol/L respectively, $p = 0.03$). There was a slight but significant decline of total serum cholesterol level with increasing age ($p=0.04$). The decrease was 0.042 mmol/l per decade. The decrease in triglyceride level with age was more prominent, 0.139 mmol/l per decade ($p<0.001$).

Between groups with different severity of atherosclerosis, patients, who did not have plaques, had significantly lower cholesterol levels compared to those with any degree of internal carotid artery stenosis. Serum cholesterol level did not differ significantly among groups with mild, moderate to severe internal carotid artery stenosis or occlusion.

Patients with internal carotid artery occlusion had higher serum triglyceride levels, compared to patients without occlusion, although because of the high standard deviation it did not reach the level of significance. Serum triglyceride levels of groups with no, mild and moderate to severe internal carotid artery stenosis were in the same range.

The univariate analysis found that age ($p<0.001$), gender ($p<0.001$), hypertension ($p<0.05$), cholesterol ($p<0.01$), triglyceride ($p<0.05$) and smoking ($p<0.001$) differed significantly among the four groups. In the complete model ordinal logistic regression age, gender, smoking and total cholesterol level were independent predictors of internal carotid artery atherosclerosis. The results were similar, when smoking was excluded from the model.

However in the third model, where gender was excluded, total cholesterol level lost statistical significance.

4.3. Parameters predicting the outcome of acute stroke

A cohort of 346 acute ischemic stroke patients (170 survived and 176 deceased) was examined. The average age of the survivors was 71.5±11.6 (mean±SD), and the non survivors was 71.5±11.4 (mean±SD). The causes of death were pneumonia (49%), cardiac failure (10%), acute myocardial infarct (6%) and pulmonary embolism (35%). The laterality of stroke did not influence the risk of death.

Based on results from the series of simple analyses, the starter multiple model had diabetes mellitus (Hazard Ratio: 1.70), serum glucose (HR: 1.06) infarct size (HR: 1.39), cardiac abnormalities (HR: 1.80), aspirin therapy (HR: 0.28), white blood cell count (HR: 1.24,), hematocrit (HR: 1.19), hemoglobin (HR: 0.80) and platelet count (HR: 1.16) as explanatory variables. In addition to these, only systolic blood pressure slope and slope squared was found to be an important predictor.

The presence of hypertension and blood pressure at admission was not an independent predictor of death. In the survivor group the systolic blood pressure on admission was ≤220 mmHg in all cases, while in the deceased group only four patients had higher than 220 mmHg (data not shown). A 1 mmHg increase/day in the systolic blood pressure was associated with a 1.07 times increase in risk of unfavorable outcome. Using the square of the systolic BP slope, the risk of fatal outcome was the lowest if the decrease of blood pressure was 2.7 mmHg/day. More expressed decrease or increase of the slope of BP change/day was associated with increased risk of fatal outcome. Considering that at admission the systolic blood pressure was approximately 160 mmHg and the ideal decrease is 2.7 mmHg/day, the optimal systolic blood pressure is approximately 140 mmHg by the end of the first week.

Cardiac abnormalities, especially ischemic heart disease and heart failure significantly worsened the outcome, with a 1.86 times risk increase of fatal outcome. One mmol/l increase of serum glucose level was coupled with an 1.11 fold increase while 50 G/l increase in platelet count with a 1.15 fold increase of the risk of death. Aspirin treatment significantly decreased the risk of death.

4.4. Factors influencing hemorrhagic transformation in ischemic stroke

All of our acute ischemia stroke patients (n=245) had brain CT at admission excluding hemorrhage or tumor. None of the patients received thrombolytic therapy and anticoagulant with therapeutical dose. The autopsy revealed ischemic infarct in 175 (71%) and ischemic infarct with HTr in 70 (29%) patients. Mean age was 71.5±11.4 years (mean±SD) and 74.8±10.2 years (mean±SD) respectively ($p>0.05$). The causes of death were pneumonia (45%), cardiac decompensation (22.5%), acute myocardial infarct (5.8%), tonsillar herniation (3.3%) and pulmonary embolism (23.3%), which did not differ between the two groups. The severity of carotid atherosclerosis ipsilateral to the stroke did not differ between patient with ischemic infarct and HTr.

Based on results from the series of simple analyses, the starter multiple model had age, sex, diabetes, alcohol consumption, presumed cardiac embolism, and infarct size as explanatory variables. In addition to these, only hypertension (binary variable) was found to be an important predictor. Significant interaction between age and embolic origin was confirmed.

Advanced age involves elevated HTr odds in patients whose cerebral infarcts are possibly or probably of embolic origin: these patients are characterized by extra 7% odds with each year increase, or a doubling of HTr odds every 11 years. On the other hand, age does not seem to be a risk factor in patients whose infarcts were not considered to be of embolic origin.

An embolic pathway itself does not necessarily mean a higher risk of HTr, a suitably high age is a prerequisite for that effect to manifest. From 75 years of age and beyond, the effect is more and more sizeable and significant as age advances.

Serum glucose level at admission and before death did not differ between non HTr and HTr patients (7.3±3.4 vs. 8.5±3.2 mmol/l and 8.5±4.7 vs. 9.5±4.9 mmol/l respectively). Diabetes mellitus in the case history incurs substantial triple odds, while alcohol seems to have a weak protecting effect, especially as far as the width of its confidence interval is concerned. Systolic and diastolic blood pressure did not significantly differ between non-HTr and HTr patients at admission, on the first, third and the seventh day. The presence of hypertension (verified with the autopsy of the heart) in the case history does not effect the risk of HTr. Larger infarcts were accompanied with three times odds for HTr than smaller ones.

4.5. Evaluation of intracranial hemodynamics in severe ischemic stroke patients

The mean a CRC in the lethal outcome group was significantly less (0.23 ± 0.08, mean ± SD) than in survivors (0.36 ± 0.09, $p < 0.03$). The mean water content in the affected hemisphere (84.85 ± 11.38%, mean ± SD) was significantly higher than in the nonaffected hemisphere (73.64 ± 11.07%, $p < 0.03$). A significant correlation was observed between the aCRC and the water content of the brain samples, both in the affected ($r=0.88$, $p<0.0001$) and in the nonaffected ($r=0.51$, $p<0.02$) hemispheres.

4.6. Cerebral effects of a single dose of vinpocetine in chronic ischemic stroke patients

The mean blood flow velocity in the supplying territory of the MCA in the symptomatic hemisphere slightly decreased following vinpocetine treatment (57.1 ± 6.3 cm/sec vs. 55.8 ± 6.2 cm/sec), whereas it increased somewhat in the contralateral MCA (56.6 ± 3.6 cm/sec vs.

58.7 ± 5.7 cm/sec). The PI increased in both the entire symptomatic and entire contralatral hemispheres, but the changes in the symptomatic hemisphere's MCA were higher (22.3 ± 9.6%) compared with that of the contralateral hemisphere (8.3 ± 6.6%; $p = 0.07$). However, none of these changes were statistically significant. The ratios between symptomatic and contralaterial hemispheres followed these trends: The velocity ratios decreased after vinpocetine treatment (from 0.99 ± 0.08 to 0.91 ± 0.07, whereas the ratio related to the PI increased (from 1.14 ± 0.13 to 1.22 ± 0.13).

5. Discussion

5.1. Cerebrovascular reserve capacity in patients with hyperlipidemia

In our study we could not demonstrate any difference in cerebral vasodilatory responses of hyperlipidemic patients as compared to healthy controls.

Similarily, Rodriguez at al. found normal cerebrovascular reactivity in thirteen of fifteen patients with long-lasting, severe familial hypercholesterolemia. Previous animal experiments and human neuropathologic investigations failed to detect any microangiopathy in hypercholesterolemia or in experimentally induced hyperlipidemia. The result of a human neuropathological study suggests, that there is positive association between serum total cholesterol concentration and atherosclerosis of basal cerebral arteries, but smaller arteries are not involved. Based on this, at least one reason of our results can be that hypercholesterolemia damages the larger cerebral arteries with the similar size of coronary arteries, while decrease of cerebrovascular reserve capacity is related to arteriolar functional impairment.

On the other hand, the impact of hypercholesterolemia on cerebral atherosclerosis could have been related to the duration of the disease. Note that duration of hypercholesterolemia is difficult to define, as it may remain asymptomatic until the first clinical signs appear or is discovered by routine laboratory screening. In our sample age of the patients was young, hyperlipidaemia was mild and probably with short disease duration. This is a possible explanation for unaltered cerebral microvascular function in hyperlipidemic patients. In humans lipid deposits and atheromas appear approximately after 20 years of hypercholesterolemia in atherosclerotic lesions. However, these changes have been observed only in larger elastic arteries (coronary and elastic arteries) and not in cerebral arterioles.

5.2. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery

Based on our study, hypercholesterolaemia seems to be an independent risk factor of internal carotid artery atherosclerosis.

In this study age was the most significant risk factor for carotid atherosclerosis. Previous studies also found not only increasing atherosclerosis but plaque progression with age. The worsening of atherosclerosis with age is probably the effect of the longer exposure to risk factors.

Male gender is also a significant risk factor of atherosclerosis in our cohort, and also in previous reports. One possible reason is the pre-menopausal hormonal difference between genders. However, although rat experiments found estrogen to be protective in focal brain ischemia, large studies on post-menopausal woman did not confirm the effect of hormone therapy in primary and in secondary stroke prevention. The other possible reason is the different prevalence of stroke risk factors between genders. In our cohort smoking was more frequent in men (38.9%) than in women (18.4%).

Smoking is also a strong predictor a plaque prevalence and internal carotid artery stenosis in our cohort and also in previous studies. The effect of smoking is multifactorial. It alters haemostatic factors, blood lipids, accelerate atherosclerosis, and changes endothelial function.

In our large acute ischemic stroke cohort, patients with normal carotid ultrasound examination had lower total cholesterol level than those who had plaques, while patients with internal carotid occlusion had higher level of triglyceride in the univariate analysis.

Although significant correlation was reported between higher level of cholesterol with increasing carotid artery stenosis, we did not find similar correlation. It is possible that the role of hypercholesterolemia in atherogenesis changes with the progression of atherosclerosis.

In the artery wall the extracellular matrix, which may have different affinities for cholesterol rich lipoproteins changes, cholesterol rich lipoprotein binding to extracellular matrix decreases with the progression of atherosclerosis. Serum triglyceride level was not an independent risk factor of atherosclerosis in the carotid artery in any of the three models.

Although hypertension is a strong risk factor for stroke and the majority of the studies found hypertension as an independent predictor of atherosclerosis our results did not find an association between hypertension and the degree of carotid artery disease. The possible reasons are that we defined hypertension based on the anamnesis, hypertension was treated and the exact duration of hypertension is usually not known.

5.3. Parameters predicting the outcome of acute stroke

Our most important finding is that in acute ischemic stroke the blood pressure should be mildely decreased even if it is not severily high (around 160 mmHg).

Majority of previous studies measured the blood pressure only at admission, so there is no study available in the literature, which assessed the relationship between outcome and the trend of blood pressure changes during the first week of acute stroke. We examined the outcome as survivor or deceased, and did not intend to evaluate the functional outcome of survivors.

Our result showed association of aspirin treatment with the reduction of death, which is consistent with previous results, where early death reduction was found in the aspirin treated group.

Blood-pressure management in acute stroke is a matter for continuing research. In acute stroke patients with higher initial blood pressure a mild decrease during the first week to 140 mmHg will result in better outcome. Patients with more and less expressed change in systolic blood pressure have less chance to survive the acute stroke. There is an increasing

body of evidence, that high blood pressure is associated with increased rate of death and dependency in acute stroke. SPECT and Doppler ultrasound studies found that modest reduction in blood pressure does not result in decrease of cerebral blood flow. Moreover, early recurrence and death resulting from cerebral edema is independently associated with high systolic BP and brain edema is less frequent in patients with a greater drop in blood pressure.

Ischemic heart disease and cardiac failure meant a 1.7-2 times risk, while atrial fibrillation 1.4 times risk to death in the simple model. Altogether the presence of cardiac abnormalities highly significantly increased the risk of death in the multiple model. These results are similar to previous reports, where atrial fibrillation, cardiac failure and myocardial infarct was associated with increase risk of death after stroke. On one hand, cardiac failure and atrial fibrillation reduces cardiac output, and besides causing larger infarct it may further compromise the ischemic, viable brain tissue. On the other hand, patients with atrial fibrillation and ischemic heart disease or cardiac failure has higher chance for cardiac death.

Serum glucose elevation increases the risk of case fatality similarly to previous studies. Major causes of worse outcome in hyperglycemia are tissue acidosis, increased blood-brain barrier permeability, impaired cerebral metabolism and increased edema.

Based on our result increased thrombocyte count is coupled with higher rates of death. This seems to differ from other studies, where lower platelet count increased the risk of death and dependency. However these studies obtained platelet count within 48 h after cerebral infarction, while in our study it was measured within 1 hour after admission. In our cohort, later the platelet count was lower in patients who died, but because of the many missing data it was not entered in the models. The probable mechanism is that platelets accumulate in the infarct and penumbra area. This may increase the infarct size, the severity of neurological symptoms and results in poor clinical outcome.

5.4. Factors influencing hemorrhagic transformation in ischemic stroke

The independent risk factors of HTr are cardioembolic stroke over the age 75 years, diabetes mellitus and the size of the infarct. In our patients without cardioembolic infarct, the age was not an independent predictor of HTr. However, we found that age is a risk factor of HTr in embolic infarct, but only in patients elder than 75 years. It is similar to the results of Okada *et al*., where patients with cardioembolic stroke elder than 70 years had higher risk of HTr compared to the younger ones. Although cardiac emboli frequently dissolve during the first days after ischemic stroke it is resulting not only in parenchymal but also in vessel wall damage. The possible explanation is that reperfusion/hyperperfusion and restoration of blood pressure might result in further damage or rupture of the microvascular endothel and this effect is amplified by advanced age.

In rt-PA treated patients diabetes mellitus, hyperglycemia were found to be a risk factor for HTr. In our cohort the serum glucose level was slightly higher in HTr patients, but not an independent predictor of HTr similarly to earlier reports without and with rt-PA therapy. However, the co-existing diabetes mellitus tripled the risk of HTr. We assume, that not the acute, but the chronic elevation of blood glucose level increases the risk for HTr. In plaques of patients with diabetes the apoptosis of vessel wall smooth muscle is enhanced, and the amount of vascular smooth muscle cells decreased. The impaired collagen synthesis, and the increased activity of matrix metalloproteinases might damage the endothelial basal lamina resulting in further damage of the vessel wall. In diabetes, the activity of endogenous anticoagulants is also decreased while coagulants and plasminogen activator inhibitor-1 increased. These pathological changes might result in diffuse damage of the microvasculature and further increase of infarct volume with higher risk of HTr.

Our finding, a mild protecting effect of alcohol consumption against hemorrhagic transformation should be evaluated with caution. The information regarding alcohol

consumption was provided by the relatives, and both the amount and the frequency are usually under- or overestimated and the reliability is questionable.

Chronic hypertension is believed to be a risk factor for HTr however large acute stroke trials did not confirm these observation. Our result confirms that hypertension does not increase the risk of HTr in acute ischemic stroke.

5.5. Evaluation of intracranial hemodynamics in severe ischemic stroke patients

An increased affected-to-nonaffected side asymmetry in CRC in the acute phase of stroke was accompanied by a significant increase in water content. Our results suggest that the compression effect of edema on the arterioles may play an important role in the acute phase of stroke. In case of sever head injury the increased intracranial pressure significantly correlates with the haemodynamic parameters measured with TCD. After the operation of subdural haemorrhage, the pulsatility index and the cerebral blood flow (CBF) velocity improves. In acute stroke, the increased intracranial pressure increases the PI and decreases the CBF, which improves after treatment to decrease edema. Besides this, the edematous change of walls of arterioles, the potassium efflux and sodium influx (with water influx) can result in vasoparalysis and decreased vasoreactivity: the severity of brain edema correlates with the grade of decrease in CRC. Our results suggest the prognostic importance of the CRC measurement in severe stroke patients.

5.6. Cerebral effects of a single dose of vinpocetine in chronic ischemic stroke patients

The symptomatic hemisphere of chronic stroke patients reacted differently to vinpocetine than vessels in healthy tissue.

The two important parameters analyzed during TCD measurements on stroke patients are usually blood flow velocity and PI. Both parameters can be influenced by several factors, including resistance in the distal vasulature, cariac contractility, aortic insufficiency, increased intracranial pressue, cerebral micronagiopathy, and so forth. In general, the PI will be higher with increased distal vascular resistance and lower with increased distal vascular resistance and lower with decreased distal vascular resistance.

Vinpocetine infusion caused an increase in the PI in both hemispheres, with a trend for the higher increase to be in the entire symptomatic hemisphere (22.3 ± 9.6%) than in the entire contralateral one (8.3 ± 6.6%). Several factors influencing the PI (cardiac contractility, aortic in sufficiency, increased intracranial pressure, cerebral microangiopathy, etc.) did not change before and after therapy, therefore it is likely that the changes experinced are caused by the drug under investigation. If the interhemispheric differences were confirmed by subsequent studies, we would speculate that vinpocetine treatment resulted in an increased peripheral resistance (higher arteriolar vascular tone), and the higher vascular resistance might result in a slight velocity decrease in the entire symptomatic hemisphere (-7.5 ± 5.6%) and a blood redistribution toward the entrire contralateral hemisphere with a slight increase of blood flow velocity (+1.6 ± 5.3%).

6. Summary

The duplex and transcrainal ultrasound examination is an simple and non invasive method which can be combined with other examinations (PET, SPECT, *post mortem*).

Based on our results the provocational TCD examination of the intracranial arteries in hypercholesterolaemia is not necessary. The B-mode ultrasound examination of the carotid arteries confirmed the role of hypercholesterolaemia in the plaque formation of these arteries. The carotid ultrasound is important in the screening and follow up of hyperlipidaemic patients, and in the cerebrovascular diagnostics and stroke prevention.

In acute ischemic stroke a mild decrease of the blood pressure to 140 mmHg by the end of the first week is associated with the highest change to survive. This observation does not confirm the previous recommendation, to decrease blood pressure only if it is higher than 220 mmHg. Because of this, further studies are needed.

Combining the TCD and pathological examination, we find correlation between cerebrovascular reserve capacity and the severity of oedema in acute stroke. As the severity of the oedema influences the outcome, the examination of the CRC may have prognostic significance.

Based on our results the risk of haemorrhagic transformation is the embolic stroke over the age 70 years, the size of the infarct and diabetes mellitus. If these disorders are present, anticoagulation therapy should be introduced cautiously.

The TCD examination may be useful to monitor the effects of drugs that influences cerebral blood flow (e.g. vinpocetine). PET examination of chronic stroke patients found increased glucose extraction especially in the surviving brain tissue, which can partly be the result of the redistribution of blood flow evaluated by TCD.

7.1. Az értekezés alapját képező teljes terjedelmű közlemények

1. Kerenyi LL, Fulesdi B, Ficzere A, Kaposzta Z, Valikovics A, Paragh G, Csiba L. Cerebrovascular reserve capacity in patients with hyperlipidemia. *J Clin Ultrasound* 2000 Mar;28(3):115-21. IF: 0,596
2. Settakis G, Molnar C, Kerenyi L, Kollar J, Legemate D, Csiba L, Fulesdi B. Acetazolamide as a vasodilatory stimulus in cerebrovascular diseases and in conditions affecting the cerebral vasculature. *European Journal of Neurology* 2003;10(6):609-620 IF: 2,000
3. S. Szakall, I. Boros, L. Balkay, M. Emri, I. Fekete, L. Kerenyi, S. Lehel, T. Marian, T. Molnar, J. Varga, L. Galuska, L. Tron, D. Bereczki, L. Csiba, B. Gulyas. Cerebral effects of a single dose of intravenous vinpocetine in chronic stroke patients: a PET study. *J Neuroimaging* 1998;8(4):197-204. IF: 1,044
4. Gulyas B, Bonoczk P, Vas A, Csiba L, Bereczki D, Boros I, Szakall S, Balkay L, Emri M, Fekete I, Galuska L, Kerenyi L, Lehel S, Marian T, Molnar T, Varga J, Tron L. Egyszeri vinpocetin-infúzió agyi anyagcserére gyakorolt hatásának vizsgálata territoriális típusú ischemiás stroke-ot szenvedett betegeken. *Orvosi Hetilap* 2001 Mar 4;142(9):443-9
5. L. Csiba, A. Valikovics, B. Fulesdi, A. Ficzere, L. Kerenyi: Non-invasive diagnosis of impairment of cerebral hemodynamics in patients with stroke risk factors. In: *New trends in cerebral hemodynamics and neurosonology*, ed: J. Klingerhoffer and E. Bartels, Elsevier Science Amsterdam 1997;576-583.
6. Káposzta Z, Kerenyi L, Bereczki D, Fekete I, Valikovics A, Fülesdi B, Csiba L. Evaluation of intracranial hemodynamics in severe ischemic stroke patients. In: *New trends in cerebral hemodynamics and neurosonology*, ed: J. Klingerhoffer and E. Bartels, Elsevier Science Amsterdam 1997;266-270.

7. Kerényi L, Mihálka L, Csiba L, Bacsó H, Bereczki D. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery. *J Clin Ultrasound* közlésre elküldve
8. Kerényi L, Kardos L, Szász J, Szatmári S, Bereczki D, Mihálka L, Hegedűs K, Csiba L. Blood pressure in the first week of ischemic stroke: its role in predicting outcome. *Stroke* közlésre elküldve
9. Kerényi L, Kardos L, Szász J, Szatmári S, Bereczki D, Hegedüs K, Csiba L. Factors influencing hemorrhagic transformation in ischemic stroke. A clinicopathological comparison. *Cerebrovasc Dis* közlésre elküldve

7.2. Az értekezés témájával kapcsolatos kongresszusi összefoglalók

1. L. Kerenyi, Z. Kaposzta, A. Ficzere, B. Fulesdi, A. Valikovics, L. Csiba. Cerebrovascular reserve capacity in patients with hypercholesterolemia. Abstract. *Cerebrovascular Diseases* 1996;6(suppl 3):43.
2. L. Csiba, A. Valikovics, B. Fulesdi, A. Ficzere, L. Kerenyi. Non-invasive diagnosis of vascular damage in patients with stroke risk factors. Abstract. *Cerebrovascular Diseases* 1996;6(suppl 3):46.
3. Z. Kaposzta, L. Kerenyi, D. Bereczki, I. Fekete, J. Kollar, J. Sikula, I. Uzonyi, A. Valikovics, B. Fulesdi, L. Csiba. Evaluation of intracranial hemodynamics in severe ischemic stroke patients. Abstract. *Cerebrovascular Diseases* 1996;6(suppl 3):47.
4. L. Csiba, L. Kerenyi, L. Galuska, L. Tron, B. Gulyas. The effect of a single dose iv. 230 mg vinpocetine on cerebral hemodynamics and metabolism. Abstract. *Agyérbetegségek* (III. Hungarian Stroke Conference) 1996;(3)12:1006.

5. Kerenyi L, Mihálka L, Csiba L, Bacso H, Bereczki D. The role of hyperlipidaemia in the atherosclerotic plaque formation of the internal carotid artery. *European Journal of Neurology* 2004;11(suppl 2):60.

7.3. Egyéb közlemények

1. Szilagyi G, Nagy Z, Balkay L, Boros I, Emri M, Lehel S, Marian T, Molnar T, Szakall S, Tron L, Bereczki D, Csiba L, Fekete I, Kerenyi L, Galuska L, Varga J, Bonoczk P, Vas A, Gulyas B.Effects of vinpocetine on the redistribution of cerebral blood flow and glucose metabolism in chronic ischemic stroke patients: a PET study. *J Neurol Sci*. 2005, Mar 15;229-230(1):275-84. IF: 2.14
2. Kerenyi L, Ricaurte GA, Schretlen DJ, McCann U, Varga J, Mathews WB, Ravert HT, Dannals RF, Hilton J, Wong DF, Szabo Z. Positron emission tomography of striatal serotonin transporters in Parkinson disease. *Arch Neurol*. 2003, Sep;60(9):1223-9. IF: 4,684
3. Aleksic S, Szabo Z, Scheffel U, Ravert HT, Mathews WB, Kerenyi L, Rauseo PA, Gibson RE, Burns HD, Dannals RF In vivo labeling of endothelin receptors with [C-11]L-753,037: Studies in mice and a dog. *Journal of Nuclear Medicine* 2001, 42 (8): 1274-1280. IF: 4,510
4. Szabo Z, Speth RC, Brown PR, Kerenyi L, Kao PF, Mathews WB, Ravert HT, Hilton J, Rauseo P, Dannals RF, Zheng W, Lee SH, Sandberg K. Use of positron emission tomography to study AT1 receptor regulation in vivo. *Journal of the American Society of Nephrology* 2001, 12 (7): 1350-1358 . IF: 6,337

7.4. Egyéb kongresszusi összefoglalók

1. Z. Kaposzta, L. Kerenyi, D. Bereczki, I. Fekete, J. Kollar, J. Sikula, Z. Regeczy, A. Valikovics, B. Fulesdi, L. Csiba. Effect of unilateral internal carotid artery occlusion on intracranial hemodynamics. Abstract. *Cerebrovascular Disease* 1996;6(suppl 3):53.
2. Z. Szabo, U. Scheffel, WB. Mathews, HT. Ravert, K. Szabo, L. Kerenyi, RF. Dannals. GA. Ricaurte. Effects of substituted amphetamines on the binding of [C-11]McN5652 in the human brain. *J Nucl Med*. 39:100P-101P, 1998.
3. Z. Szabo, K. Sandberg, R. Brown, L. Kerenyi, K. Szabo, WB. Mathews, JL. Osborn, HT. Ravert, RF. Dannals. Angiotensin II subtype 1 receptor regulation during dietary sodium manipulation. *J Nucl Med*. 39:196P, 1998.
4. Z. Szabo, RP. Brown, J. Elstrodt, K. Sandberg, L. Kerenyi, WB. Mathews, J. Hilton, U. Hamper, K. Szabo, JL. HT. Ravert, Osborn, D. de Zeeuw, RF. Dannals. Effects of Lisinopril on the expression of the AT1 receptors in renal artery stenosis. Abstract. The 2nd International Symposium on Angiotensin II Antagonism. 1998.
5. Z. Szabo, L. Kerenyi, K. Szabo, WB. Mathews, HT. Ravert, RF. Dannals. G. Wand.[C-11](+)McN5652 binding to 5-HT transporter in recovering alcoholics. *Society for Neuroscience* Abstracts. 1998;24(1):991.
6. Molnar M, Valikovics A, Gulyas B, Tron L, Kerenyi L, Mechler F, Csiba L. Changes of neuronal metabolism and cerebrovascular reserve capacity in mitochondrial disorders studies by positron-emission tomography (PET) and transcranial Doppler sonography (TCD). *Neurology* 1998, 50 (4): P01118 Suppl. 4.
7. Kerenyi L, Szabo Z, Scheffel U, Mathews WB, Ravert HT, Szabo K, Dannals RF: Assessement of serotonergic inervation with [C-11]McN5652/PET: validation of kinetic models in baboons. *J Nucl Med* 40:29P, 1999.

8. Szabo Z, Sandberg K, Brown RP, Elstrodt J, Kerenyi L, Mathews WB, Hilton J, Hamper U, Szabo K, de Zeeuw D, Dannals RT: Increased AT1 receptor expression after treatment with the ACE inhibitor lisinopril. *J Nucl Med* 40:92P-93P, 1999.
9. Yanez M, Szabo Z, Kerenyi L, Heath E, Forastiere A: Attenuation correction improves 18FDG/PET assessement of tumor response. *J Nucl Med* 40:147P, 1999.
10. G. Esposito, Z. Szabo, A. Lokesh, L. Velez, L. Kerenyi, M. Reinhardt, M. Pappagallo. Imaging of neurogenic inflammation during migraine. *Society for Neuroscience* Abstracts 1999;25(1):140.
11. O. Vaterlein, Z. Szabo, M. Samal, L. Kerenyi, U. Scheffel, GA. Ricaurte. Factor analysis of dynamic PET studies performed in healthy individuals and in MDMA users. *Society for Neuroscience* Abstracts 1999;25(1):715.
12. L. Kerenyi, GA. Ricaurte, D. Schretlen, K. Park, WB. Mathews, HT. Ravert, K. Szabo, RF. Dannals, S. Aleksic, U. McCann, Z. Szabo. Clinical features and monoaminergic deficits in idiopathic Parkinson's disease - a pet study. *Society for Neuroscience* Abstracts 1999;25(2):2114.
13. Chen MK, Aleksic S, Samal M, Kerenyi L, Vaterlein O, Ricaurte G, Szabo Z. Factor analysis of dynamic PET studies of the serotonin transporter in idiopathic Parkinson's disease. *Journal of Nuclear Medicine*, 41: (5) 546 , Suppl. 2000.
14. Aleksic S, Szabo Z, Scheffel U, Ravert HT, Vaterlein O, Kerenyi L, Gibson RE, Ryan C, Hamill T, Burns HD, Dannals RF. In vivo labeling of endothelin receptors with [C-11]L-753, 037. *Journal of Nuclear Medicine*, 41: (5) 1080 , Suppl. 2000.
15. Chintakayala DP, Aleksic S, Chen MK, Brown PR, Kerenyi L, Reinhardt M, Sandberg K, Szabo Z. Up-regulation of angiotensin AT1 receptors in the adrenal and kidney after occlusion of a renal artery. *Journal of Nuclear Medicine*, 41:(5)1362,Suppl. 2000.

16. S. Molnár, L. Kerényi, M. Ritter, T. Magyar, E.B. Ringelstein, L. Csiba. Comparison of in vivo carotid IMT and carotid wall thickness measured with a novel method (comparison of in vivo and post-mortem data). *Cerebrovascular Disease*, 16(suppl 4):75; 2003.
17. L. Kerényi, S. Molnár, M. Ritter, T. Magyar, E.B. Ringelstein, L. Csiba. Correlation of atherosclerotic changes between carotid, coronary and femoral arteries. Comparison of in vivo findings with autopsy. *Cerebrovascular Disease*, 16(suppl 4):54; 2003.
18. J. Konya, L.L. Kerenyi, S. Molnar, T. Magyar, M. Ritter, B.E. Ringelstein, L. Csiba. The relationship between the intima-media thickness (IMT) of the carotid artery and the atherosclerosis of the carotid, femoral and coronary arteries and the role of chlamydia pneumoniae in the atherosclerosis of the extra-and intracranial arteries. *European Journal of Neurology* 2003;10 (suppl 1) 46.
19. Kerenyi L, Molnar S, Ritter M, Magyar T, Csiba L. Comparison of in vivo carotid IMT and post mortem measured atherosclerotic changes of carotid, coronary and femoral arteries. *Stroke* 35 (6): E258-E258 2004.