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

Neurosonological investigations in stroke risk factors

By

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1. Justification of the research subject

Stroke is the third leading cause of death and the most important cause of long term disability in Western countries. Despite the advent of treatment of selected patients with acute ischemic stroke, the best approach to reducing the burden of stroke remains prevention. Effective strategy for the primary and secondary prevention of stroke includes screening and treatment of risk factors.

Risk factors for a first stroke were classified according to potential for modification. Nonmodifiable risk factors are the age, sex, ethnicity and positive family history of stroke. Modifiable risk factors are the hypertension, smoking, diabetes mellitus, carotid artery stenosis, hyperlipidemia, atrial fibrillation and other cardiac disease. On the other hand, these conventional risk factors are associated with only about 50% of cases of cardiovascular diseases, consequently, other factors can play role in atherogenesis.

We investigated patients with modifiable stroke risk factors, such as *hypertension*, *hyperlipidemia* and *occlusive carotid artery disease*. Our examinations were performed on *young subjects*. Using the relatively simple, non-invasive *neurosonological methods* we would like to detect the early stage of atherosclerosis, and we were looking for factors that might differentiate young patients with carotid atherosclerosis from those without early atherosclerotic changes in several aspects.

2. Aims of the studies

2.1. Carotid bruits and results of carotid duplex ultrasound

Extracranial carotid artery occlusive disease is the major risk factor of the ischemic stroke. In symptomatic high-grade carotid artery stenosis, endarterectomy is the method of choice to prevent stroke recurrence. Carotid duplex sonography is a noninvasive technique available nowadays in many centers. This technique allows detection and quantification of extracranial occlusive carotid artery disease.

Carotid bruits identified on neck auscultation by means of a stethoscope have repeatedly been described to be associated with high-grade carotid artery stenosis. In one study the sensitivity of the detection of carotid bruits for the presence of a ≥50% stenosis was 79% and specificity

was 65%. Another study, however, confirmed carotid artery stenosis of ≥50% in only 16% of patients with a carotid bruit.

- The present study addresses the question whether in patients at risk of carotid stenosis it is still worthwhile to auscultate the neck or whether the physician should proceed directly to ultrasound.

2.2. Functional transcranial Doppler monitoring in young hypertensives

Hypertension is a major risk factor for both cerebral infarction and intracerebral hemorrhage. The control of high blood pressure contributes to the prevention of stroke as well as to the prevention and reduction of other organ damage. Chronic hypertension results in vessel wall remodeling in hypertensive patients causing impaired cerebral hemodynamics and autoregulation. In the early phase of hypertension cerebral blood flow velocity does not differ significantly from values measured in controls. Therefore, in order to detect dysfunctional vasoregulation - in hypertension and in other stroke risk factors -, different tests eliciting either vasodilation or vasoconstriction (CO_2 stimulation, acetazolamide-test, hyperventilation) were used to investigate cerebral hemodynamics. As the impairment of vasoreactivity is assumed to increase the risk of stroke, it is important to use a sensitive provocation test to detect early changes.

Recently ergometer cycling combined with continuous transcranial Doppler (TCD) monitoring proved to be useful for the evaluation of cerebral hemodynamics in normal subjects.

- Our aim was to apply this *physiological test on recently diagnosed non-treated hypertensive subjects*. We assumed that arteriolar dysfunction occurs early in the course of hypertension, and that dysfunction could be detected by measuring changes in flow velocity in the middle cerebral artery during physical exercise.

Other studies suggested that adequate antihypertensive therapy may normalize impaired cerebral blood flow autoregulation in the early stage of chronic hypertension. 19 out of 30, previously investigated non-treated hypertensive patients could be evaluated again after 3-year antihypertensive treatment.

- The aim of this study was to investigate the effect of 3-year antihypertensive treatment on cerebral hemodynamics in hypertensives using the same method, TCD combined with ergometer cycling.

2.3. Measurement of common carotid artery intima media thickness (IMT) in young hyperlipidemic patients

Increased IMT is an early marker of the atherosclerotic process and prospective studies have shown that increased IMT of the common carotid artery is a powerful predictor of coronary and cerebrovascular complications. The association of carotid artery IMT with the well-known cardiovascular risk factors, such as age, smoking and hypertension, has been demonstrated in several studies, and IMT was linearly related to the total number of cardiovascular risk factors. The role of serum cholesterol and triglyceride in carotid atherosclerosis is unclear. Recent population studies came up with conflicting conclusions. In the Muscatine study total cholesterol as well as triglycerides was associated with IMT both in men and women. In the Tromso study total cholesterol was an independent predictor of IMT in both sexes, whereas triglyceride levels were independently associated with IMT only in women. In contrast with these two reports, no correlation was found between total cholesterol and IMT in an analysis from the Framingham study.

- For these reasons we felt that the question whether cholesterol and triglyceride do or do not have an effect on IMT has not been completely settled and we assumed that analyzing a *wide range of lipid values* could help to resolve these conflicting conclusions. To minimize the confounding effect of age, one of the strongest predictor of IMT as much as possible, we chose *young subjects* (mean: 44 years, all subjects below 55 years of age) for this study.

2.4. Possible risks of early occlusive carotid artery disease

Consequences of atherosclerosis like ischemic heart and cerebrovascular diseases rank among the most important public health issues. Increased IMT was reported to occur in an earlier phase of the atherosclerotic process. Most publications on carotid artery disease focus on elderly subjects and in a population study of over 16.000 people 90% of subjects affected with severe carotid lesions were over 55 years of age. Age is the primary risk factor for carotid artery disease and examining an elderly patient group may mask the effects of risk factors of early atherosclerosis. Therefore in the present study we tried to identify the role of several potential risk factors of early atherosclerosis in a case control study in subjects below 55 years of age.

We were looking for factors that might differentiate young patients with carotid atherosclerosis from those without early atherosclerotic changes in several aspects. Four major alterations were anticipated:

- We expected that markers of inflammation are increased in patients with early onset carotid atherosclerosis.
- We hypothesized that the decreased resistance of low-density lipoprotein to oxidative modification is an independent risk factor for cerebral atherosclerosis.
- We expected increased IMT in young patients with occlusive carotid artery disease as IMT was reported to be significantly greater in patients with than without carotid stenosis.
- In addition we checked previously described alterations as high plasma homocysteine concentrations were reported to be associated with an increased risk of external carotid artery sclerosis in the elderly.

3. Methods

3.1. Carotid artery auscultation

Bilateral auscultation of the carotid arteries and the aortic and mitral valve was performed using a Littmann™ Select Stethoscope (USA, 3M Health Care). Carotid auscultation comprised the common carotid artery, the presumed site of the carotid bifurcation, and the submandibular internal carotid artery. Radiation of murmurs from cardiac origin was also noted.

3.2. Color-coded carotid duplex ultrasound

Carotid arteries were investigated by color duplex ultrasound (7.5MHz linear probe, Sonos 2500 in Münster, and Sonos 2000 in Debrecen, Hewlett Packard, Palo Alto, CA, USA). The investigation included longitudinal and transverse examinations of the carotid arteries. Both diameter and area reductions were measured and calculated at the site of maximal stenosis in the extracranial internal carotid arteries (ICA) according to the European Carotid Surgery Trial (ECST) method. Furthermore, the peak systolic, mean and end-diastolic flow velocities in the common carotid artery and in the jet of the ICA stenosis were recorded (angle-adjusted). For screening, the ICA stenosis was classified in categories of 10% according to international criteria. Plaques with stenosis <50% were classified according to their lumen reduction on B-mode. A peak systolic velocity of at least 120 cm/s was the threshold for a stenosis of 50%. In the case of indirect haemodynamic criteria, the stenosis was classified to have 80%. Occlusion was diagnosed in the complete absence of detectable flow in and above the stenosis and the presence of corresponding indirect hemodynamic criteria. Subjects were also examined by transcranial Doppler (2MHz probe, Multidop X, DWL, Sipplingen, Germany) including the intracranial segments of the internal carotid arteries. Periorbital arteries were investigated by continuous-wave Doppler (8MHz probe, Multidop X, DWL).

3.3. Intima-media thickness (IMT) measurements

Online measurements of IMT were performed in both common carotid arteries (CCA) at about 10 mm proximal to the carotid bulb. IMT was measured between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (upper layer of the adventitia) in the far (deeper) artery wall. All measurements were performed on frozen enlarged B-mode images (factor:2) at the end of a heart cycle (end-diastole), and the

transducer was in the medio-lateral directions. The mean value of the 2 sides (mean online IMT) and the larger of these two values (max online IMT) were recorded in all cases.

Offline analysis of CCA IMT was made on video images based on the Atherosclerosis Risk in Communities (ARIC) study protocol. The IMT was measured in the far wall of both CCA-s on the 1-cm segment proximal to the dilatation of the carotid bulb. In each of this 1-cm segment, 11 measurements of IMT were performed at 1-mm increment. Therefore for each patient the mean of the 22 IMT values (11 each side) could be calculated. This mean value was defined as the mean offline IMT. In hyperlipidemic study, an independent ultrasonographer, blinded to the characteristics of study subjects, repeated the offline analysis in all 232 vessels.

3.4. Transcranial Doppler combined with ergometer cycling

Ergometer cycling was performed by Ergocomp 2000 (Labtech Kft., Hungary) according to the WHO protocol. The loading was increased by 25 watts every second minute up till the submaximal heart rate [i.e. 0.85(220-age)] or up till 220 mmHg systolic blood pressure was reached. The test was discontinued when any complaints, like chest discomfort, dizziness, or ST-segment depression greater than 0.2 mV, or tachyarrhythmia appeared. Electrocardiogram (ECG), heart rate, end-tidal CO_2 ($etCO_2$) (Novametrix, US) were continuously monitored, blood pressure (BP) was measured by cuff. Mean blood flow velocity (MV) in the middle cerebral artery (MCA) was registered continuously during ergometer cycling by Multidop X (DWL, Sipplingen, Germany) with 2 MHz probes fixed bilaterally over the temporal bone. To relate the changes of MV to the simultaneous changes of $etCO_2$, the ratio of $\Delta MV/\Delta etCO_2$ was calculated.

3.5. Blood sampling

Homocysteine was determined by the Abbott Axsym system (Abbott Laboratories, Abbott Park, IL, USA). Hematological investigations were performed by the Sysmex SF-3000 (TOA Medical Electronics Co, Ltd, Kobe, Japan) automated hematology analyzer. Fibrinogen was measured by the Clauss method using an ST-A Compact coagulometer (Stago, Asnieres, France). Serum lipids and C-reactive protein (CRP) were measured on the Cobas Integra Analyser (Roche, Basel, Switzerland). We utilized a microassay, based on the kinetics of heme-catalyzed lipid peroxidation of LDL, to assess the resistance of lipoprotein to oxidative modification.

4. Subjects

4.1. Carotid bruits and results of carotid duplex ultrasound

145 patients, aged from 23 to 87 years were investigated (mean 62±14 years) at the Department of Neurology in Münster. There were 66 women and 79 men. In 17 patients only one carotid artery was auscultated. The auscultations were done unaware of the patient's history and of the result of the ultrasound investigation by an experienced medical doctor, who did not perform the ultrasound investigations.

4.2. Functional transcranial Doppler monitoring in young hypertensives

a. Thirty non-treated essential hypertensive patients (7 women, 23 men, age: 42.4±5.4, range 35-53 years) and thirty, age and gender matched, healthy volunteers (8 women, 22 men, age: 40.6±8.3, range 22-50 years) were investigated by transcranial Doppler combined ergometer cycling.

Hematocrit, hemoglobin, white cell and platelet counts, and serum or plasma levels of cholesterol, triglycerides, fibrinogen and glucose were evaluated. Patients with pathological laboratory values, as well as those who had any previous history suggesting neurological or heart disease were excluded. We also excluded subjects who had either neurological symptoms on physical examination, or signs of ischemic cardiac disease and/or arhythmia on resting ECG, or significant carotid artery stenosis on duplex scanning. Because of the exhausting of patients during physical exercise we used the 6-minute loading data for analysis.

b. Out of 30 non-treated hypertensive patients 19 patients (3 women, 16 men, age: 45.8±5.4 years) could be investigated again three years later using the same method. Eleven out of nineteen were regularly treated (eight subjects took ACE-inhibitors, one β-blocker, one Ca-antagonist and one was treated by ACE-inhibitor and β-blocker) and eight patients did not take their antihypertensive medications due to compliance problems. Patients in treated group did not take their medications on the day of physical exercise. Because of the exhausting of patients during physical exercise we used the 2-minute loading data for analysis. The absolute changes of hemodynamical parameters were calculated as increase of values, measured at rest. To relate the changes of MV to the simultaneous changes of $etCO_2$, the ratio of $\Delta MV/\Delta etCO_2$ was calculated.

4.3. Measurement of common carotid artery intima media thickness (IMT) in young hyperlipidemic patients

Patients were recruited at the Lipid Outpatient Service and at the Department of Neurology, University of Debrecen. The upper age limit was set at 55 years (mean age: 44.1±8 years). After getting information about the purpose of the study the participants signed a consent form before blood sampling and ultrasound examination with measurement of common carotid artery IMT. A detailed risk factor questionnaire was completed including the maximal serum cholesterol and triglyceride values in the history as documented in medical records. Based on the highest cholesterol and triglyceride values in the case history, the type of hyperlipidemia was defined according to the recommendations of Ginsberg and Goldberg. In brief, subjects were divided into the following subgroups:

- isolated hypercholesterolemia (N=20): cholesterol > 6.5 mmol/L, triglyceride ≤ 2.8 mmol/L;
- isolated hypertriglyceridemia (N=12): triglyceride > 2.8 mmol/L, cholesterol ≤ 6.5 mmol/L;
- combined hyperlipidemia (N=28): cholesterol > 6.5 and triglyceride > 2.8 mmol/L;
- control group (N=30): cholesterol ≤ 5.2 and triglyceride ≤ 1.7 mmol/L;
- borderline hyperlipidemia (N=26): 5.2 < cholesterol ≤ 6.5 mmol/L and/or 1.7 < triglyceride ≤ 2.8 mmol/L.

4.4. Possible risks of early occlusive carotid artery disease

Patients were recruited at the Neurosonological Laboratory of the Department of Neurology, University of Debrecen. The upper age limit was set at 55 years. Those with an at least 30% internal carotid artery stenosis or occlusion at screening were invited to participate in the study. 20 patients had internal carotid artery occlusion (mean age: 48.2±4.8 years), and 45 patients had ≥30% internal carotid artery stenosis (mean age: 48.2±4.2 years). An age- and gender matched control group (N=35, mean age: 47.5±5.6 years) was recruited who had no plaques or stenosis of the carotid arteries by duplex examination. After getting information about the purpose of the study the participants signed a consent form before blood sampling and ultrasound examination.

4.5. Statistical methods

Normality of parameters was checked by the Kolmogorov-Smirnov or Saphiro-Wilk tests. According to the distribution, the unpaired t-test, the Wilcoxon or Mann-Whitney test were used for comparisons between groups. Changes over time between controls and hypertensive patients, and between treated and non-treated hypertensives were compared by repeated measure ANOVA.

In the hyperlipidemic study the Mann-Whitney U-test, the Kruskal-Wallis ANOVA and the Spearman rank order correlation tests were applied. Frequencies were compared by the Pearson chi-square test. General regression models were used to look for correlates of IMT.

In the investigation of young patients with carotid artery occlusive disease two-way ANOVA was used to control for smoking in group comparisons. Quartiles of the study population were formed by homocysteine, CRP, and IMT values. The role of smoking on IMT was checked in an analysis of covariance model, where IMT was the dependent variable, smoking status was the categorical predictor and serum parameters found to differ between controls and patients were used as continuous predictors.

Statistical significance was assumed when $p < 0.05$. Statistica for Windows v. 6.1 (StatSoft, Tulsa, OK) was used for data analysis.

5. Results

5.1. Carotid bruits and results of carotid duplex ultrasound

From 33 carotid arteries out of 273, a carotid bruit not propagated from the heart was noted. There were 24 distal CCA or extracranial ICA stenoses of 50-69%, 16 stenoses of 70-99%, 14 extracranial ICA occlusions, and 4 external carotid artery (ECA) stenoses of 50-99% on ultrasound. In 25 of these arteries, a bruit was present. In 9 out of 16 patients with extracranial ICA stenosis from 70-99%, and in 6 out of 14 patients with ICA occlusion, a bruit was detected. In 1 additional patient with a middle-grade ECA stenosis, a bruit was also present. In 7 additional patients, a bruit was present in the absence of any carotid artery stenosis or goitre. Sensitivity of carotid auscultation for ≥70%-99% stenosis of the distal CCA or extracranial ICA was 56%, specificity was 91%, positive predictive value was 27%, and negative predictive value was 97%.

5.2. Functional transcranial Doppler monitoring in young hypertensives

a. 30 non-treated hypertensive patients and 30 age- and gender matched healthy subjects were investigated by transcranial Doppler monitoring during physical exercise.

Before testing systolic (SBP) and diastolic (DBP) blood pressure values were significantly higher in the hypertensive group compared to controls (SBP: 174.2±11.1 and 138.9±15.4 mm Hg; DBP: 94.1±18.1 and 77.8±3.8 mm Hg, means ± SD, $p < 0.001$).

Duration of loading differed between hypertensives and controls (4 and 7 min, $p < 0.001$).

No significant differences in MV, $etCO_2$ and heart rate could be found between the two groups before cycling. BP, heart rate, and $etCO_2$ significantly increased over time during loading, but the time course of changes was parallel in controls and hypertensives. No significant differences could be detected between the maximal absolute and relative MV, $etCO_2$, heart rate values of controls and HT subjects during the test period.

The absolute values of MV measured at 0, 2, 4 and 6 min after starting cycling increased continuously in controls but reached a plateau at around 2 minutes in hypertensive subjects.

The values of the $\Delta MV/\Delta etCO_2$ ratio which demonstrated the capacity of arteriolar vasodilation - i.e. changes in flow velocity per unit change in $etCO_2$ - seems to exhaust in hypertensive patients.

During 6 min cycling the time course of absolute values of blood flow velocity in the middle cerebral artery and that of the changes in the ratio of mean velocity/end-tidal CO_2 differed significantly between hypertensives and controls ($p = 0.03$ and $p = 0.02$, respectively).

b. 19 out of 30, previously investigated non-treated hypertensive patients could be evaluated again using the standard method after 3-year antihypertensive treatment. 11 were regularly treated (treated group), and 8 did not take their antihypertensive medications due to compliance problems (non-treated group). The mean age in the total group was 45.8 years, and there was no significant difference between treated and non-treated groups. Duration of loading did not differ between the groups (4.1±1.8 and 4.9±1.9 min).
There was no difference between treated and non-treated groups in baseline systolic BP, heart rate, MV, $etCO_2$ and PI during loading ($p=0.652$, $p=0.442$, $p=0.967$, $p=0.778$, $p=0.96$ respectively, using Mann-Whitney test). Resting diastolic blood pressure was significantly lower in treated group comparing to non-treated patients (80±3 and 89±6 mmHg, $p=0.005$).

Because of the exhausting of patients during physical exercise we used the 2-minute loading data for analysis. The absolute changes of hemodynamical parameters were calculated as increase of values, measured at rest. Significant differences could not be detected between the absolute MV, $etCO_2$ and systolic BP changes of treated and non-treated HT subjects during the test period. Increasing of heart rate was lower in treated group during the 2nd examination compared to those of before treatment.

The values of the $\Delta MV/\Delta etCO_2$ ratio measured at 2 min were significantly lower during 2nd examination compared to identical values of 1st examination i.e. the capacity of arteriolar vasodilatation - changes in flow velocity per unit change in $etCO_2$ - seems to exhaust in non-treated hypertensive patients (mean±SD: 1.53±0.98 és 2.26±0.84 cm/s/mmHg^{-1}, $p=0.04$). In treated group, the values of the $\Delta MV/\Delta etCO_2$ ratio were the same after 3-year treatment compared to values of examination before treatment (mean±SD: 1.94±1.04 és 1.71±1.03 cm/s/mmHg^{-1}, $p=0.38$).

5.3. Measurement of common carotid artery intima media thickness (IMT) in young hyperlipidemic patients

The mean age in the total group was 44.1±8.0 (mean±SD) years and there was no difference among groups (p=0.218). Of the total group of 116 subjects 27 were smokers, 39 were hypertensive, and 8 had diabetes. Three subjects had cerebrovascular disease and one had peripheral arterial disease in the history. Smokers had higher serum cholesterol than non-smokers (8.1±3.3 and 6.7±3.0 mmol/L, p=0.013) but they did not differ in IMT. Those with hypertension had significantly higher triglyceride and cholesterol values and in average 0.05 - 0.07 mm thicker IMT than normotensives. Diabetic subjects had significantly higher triglyceride and cholesterol values and a tendency for thicker intima-media than those without diabetes.

Mean serum triglyceride concentration in the hypertriglyceridemic group was over 3 times higher (5.79±5.3 mmol/L), whereas mean serum cholesterol values were close to 2 times higher in the hyperholesterolemic and combined groups than the upper values of the normal range (8.76±1.9 and 10.32±4.0 mmol/L). Mean serum triglyceride was close to ten times higher of the upper value of the normal range, in the combined hyperlipidemic group (16.5±18.6 mmol/L). Due to the skewed distribution of triglyceride values, a logarithmic transform was used in further analyses. Plasma homocysteine was somewhat higher in the combined hyperlipidemic than in the other groups, but the difference was not statistically significant among the 5 groups.

Common carotid artery IMT significantly differed among the study groups. This difference was significant when mean or maximal online readings for IMT were compared (p=0.001), as well as when offline measured mean IMT were compared among groups (p= 0.0099).

We searched for correlates of IMT in two steps. First, univariate Spearman correlation coefficients were calculated between possible predictors and the IMT data. In the next step we performed a multivariate analysis using a general regression model where age, and actually measured triglyceride, cholesterol and HDL-C values were used as continuous predictors and smoking, hypertension and diabetes as categorical factors. For the multivariate analysis we present the offline obtained IMT values. The findings were similar when IMT values of online measurements were used in the model.

From the univariate analysis it can be seen that without controlling for other factors, age, body mass index, triglyceride, cholesterol, HDL-C and LDL-C all had significant association with IMT. Although still significant, the relationship between these factors and IMT became weaker when the IMT values were measured blinded to the subjects' features. IMT readings of the 2 sonographers, on the other hand, corresponded well (R=0.822, p<0.0001). The results of the multivariate analysis revealed that age and cholesterol had strong effects on IMT (p=0.0004 és p<0.0001), whereas there is an inverse relationship between HDL-C and IMT (p=0.0106), even after controlling for factors like smoking, hypertensive and diabetic status. Triglyceride did not have an effect on IMT in this analysis.

5.4. Possible risks of early occlusive carotid artery disease

The mean age in the total group was 48 years with no difference between controls, the stenotic and occlusion groups. Men and women distributed equally in the control and stenotic groups, but most patients with occlusion of the carotid artery were men. There was no difference in the presence of diabetes, heart diseases and the family history of cerebrovascular diseases among the patient groups and controls. Hypertension was more prevalent in the stenotic subgroup than in the others. More of the patients were smokers. Ischemic stroke or TIA was more prevalent in patients than in controls. Men had significantly higher BMI (29±5 and 26±4, P=0.012), and homocysteine (13.2±6.1 and 10.2±4.9 μmol/L, P=0.003), than women. There was no gender difference in inflammatory markers or lipid parameters. No difference was found in inflammatory or lipid parameters, homocysteine and IMT between patients with and without a history of symptomatic cerebrovascular disease (P > 0.05 in all comparisons).

White blood cell (WBC) count (P<0.001), fibrinogen (P = 0.0009) and CRP (P = 0.0012) were higher in patients with occlusive carotid artery disease. Due to the skewed distribution of serum homocysteine a normally distributed natural logarithmic transform of homocysteine was also analyzed by ANOVA: patients with carotid occlusion had higher values than controls and patients with carotid stenosis (P = 0.028). Whereas lipoprotein (a) was higher in both patient groups than in controls, there were no differences among the 3 groups in triglyceride, cholesterol, HDL-C, LDL-C, Apo-A1 and Apo-B levels. There was significant difference among the three groups in IMT for online readings, as well as for mean IMT and

the patient groups had thicker intima-media layer in both comparisons (P<0.0001, and P=0.0007, respectively).

As several parameters seemed similar in the stenotic and the occlusion subgroups, a separate analysis was performed when a pooled stenotic and occlusion group was compared to the control group. Whereas levels of inflammatory markers (WBC, P < 0.0001; CRP, P = 0.0003; fibrinogen, P = 0.0002) and lipoprotein (a) (P = 0.0042) were significantly higher in patients than in controls, homocysteine did not differ from that of controls (P = 0.2). Both online measured and mean IMT values were significantly higher in patients than in controls (P < 0.001).

Smokers had significantly higher WBC (8.7±2.1 vs. 6.9±1.8 G/L), plasma fibrinogen (4.2±1.0 vs. 3.4±0.8 g/L), CRP (8.1±11.4 vs. 4.5±3.1 mg/L), lipoprotein (a) (410±486 vs. 297±469 mg/L), and online read IMT (1.30±0.33 vs. 1.05±0.26 mm) than nonsmokers. As these were the factors that were higher in patients than in controls, and more patients than controls were smokers (P < 0.001), to control for the effect of smoking a two-way ANOVA with fixed effects was used with group and smoking status as independent factors. The difference between the 3 groups remained statistically significant for WBC, ln homocysteine and online measured IMT (P = 0.01, P = 0.03, and P = 0.016, respectively) after controlling for smoking status. Smoking also had an effect on WBC (P = 0.04). After controlling for the effect of smoking, CRP was not different among the 3 groups any more. The difference in fibrinogen between groups was entirely due to the effect of smoking (group effect: P = 0.41; smoking effect: P = 0.0098). The effect of smoking on IMT was further checked in an ANCOVA model where smoking status was used as a categorical predictor, and age, WBC count, lipoprotein (a), CRP and homocysteine as continuous predictors. Of these smoking (P = 0.03) and fibrinogen (P = 0.006) were found to be significant predictors of IMT in the whole study population.

To further check whether subgroups differ based on the distribution of some features quartiles were formed of all subjects (including patients and controls) by IMT, CRP and homocysteine values. When quartiles were compared based on IMT, the subgroups differed in inflammatory markers, but not in other features like lipids or homocysteine. WBC count, fibrinogen and CRP were the lowest in the lowest IMT quartile and highest in the highest IMT quartile. The tendency was similar when quartiles were formed by the offline measured mean IMT, but the

difference among subgroups was on the margin of statistical significance (WBC count: P = 0.07, fibrinogen: P = 0.058, and CRP: P = 0.091).

No difference was found among homocysteine quartiles, including IMT, inflammatory markers and lipid parameters.

CRP quartiles significantly differed in WBC, fibrinogen, serum total cholesterol, HDL-C, lipoprotein (a), LDL resistance to oxidative stress (Δt at V_{max}), and online read IMT, with lowest values in the lowest CRP quartile for all parameters.

6. Discussion

6.1. Carotid bruits and results of carotid duplex ultrasound

The North American and the European Symptomatic Carotid Endarterectomy Trial demonstrated the benefit of carotid endarterectomy for symptomatic patients with 70-99% carotid stenosis. Therefore the group of patients, who have an ICA stenosis in that range, are actually the target group for invasive therapeutic interventions. In our study, sensitivity of carotid auscultation for ≥70%-99% stenosis of the distal CCA or extracranial ICA was 56%, specificity was 91%, positive predictive value was 27%, and negative predictive value was 97%. Because of the high negative predictive value, carotid auscultation seems to be a useful screening method for the first examination of patients.

A carotid bruit may become audible if the degree of stenosis is greater than 25%. Only a small percentage of carotid bruits are not associated with pathologic findings in the ICA by ultrasound. Other conditions may also cause neck bruits, such as venous hums and vessel kinking. A high cardiac output can be occasionally found as the reason for a bruit especially in younger patients. Furthermore, according to other study, approximately 10% of carotid bifurcation murmurs emanate from the ECA. In our study, 1 out of 33 patients with bruit had a middle-grade ECA stenosis, and in 7 out of 273 carotid arteries (2.6%) no obvious reason for bruit was detected by ultrasound. Other studies have reported bruits in the neck in approximately 4% of normal people.

On the other hand, sensitivity of carotid auscultation for severe distal CCA or ICA was 56%, 7 out of 16 patients with 70-99% carotid artery stenosis had no bruit. Out of our 7 patients with severe extracranial carotid artery stenosis without bruit, 3 had more than 90% stenosis of the ICA, 1 had a 70% distal CCA stenosis, and only 3 had a 70-80% extracranial ICA stenosis on duplex ultrasound. In other studies, about a third of patients with significant stenosis do not have carotid bruits. According to Sauve and coworkers, focal carotid bruits become more frequent with increasing degree of stenosis, peak in the 70 to 89% range, and become much less common in stenosis of more than 90%.

Theoretically, when the artery is occluded there should be no turbulence and, thus, no bruit. However, bruits are still audible in 6 out of 14 occluded ICAs (43%). Other studies reported a bruit in 21% and 24% of patients with an occluded artery. The most likely origin of a bruit in

patients with an occluded ICA is flow alteration within the carotid bulb and compensatory hyperperfusion of the ECA.

Our data show that the presence of a carotid bruit is a good clinical predictor of extracranial carotid artery disease. However, it fails to reliably depict patients with a significant carotid artery stenosis that may have therapeutic implications. The absence of a bruit on carotid auscultation can rule out stenosis in a large percentage of patients, but in patients who are suspected to have carotid stenosis, it seems inappropriate to restrict the investigation to auscultation of the neck. However, as a general clinical screening method, it is still a useful tool.

6.2. Functional transcranial Doppler monitoring in young hypertensives

Stroke occurs eight times more frequently in hypertensives than in normal subjects. One of the hypertension mechanisms causing stroke is the adaptive changes in the resistance vessels. The resistance vessels adapt themselves to increased systemic pressure by an appropriate reduction of lumen in order to keep flow constant. The wall:lumen ratio increases to allow the vessels contract against higher blood pressure. Vascular remodeling impairs the ability of vessels to dilate or constrict after different stimuli. Clinically symptom-free hypertensives with decreased vasomotor reactivity may have a higher risk for stroke. It also assumed that antihypertensive therapy may normalize impaired cerebral blood flow autoregulation in the early stages of hypertension.

Hemodynamics of hypertensives has already been investigated by PET, SPECT and TCD at rest and after stimulations. Constant cerebral arterial diameters have been found under moderate changes of mean BP and $etCO_2$ by Giller. These data suggest that the TCD velocities may closely reflect blood flow through the insonated artery. Hemodynamics of hypertensives has already been frequently investigated by TCD at rest and after stimulations. Significantly less velocity changes could be observed also in our study using ergometer cycling on hypertensive patients. Although CO_2 and acetazolamide stimulation are also useful techniques for the investigation of cerebral hemodynamics, but both of them are chemostimulants. The ergometer cycling simulates much better the physiological stresses, experienced by the hypertensives in their every-day life. The blood velocity changes in the MCA during physical exercise have already been investigated in normal subjects, but to our

knowledge, no study investigated cerebral hemodynamics of hypertensives using this technique. Our resting velocity values were similar to that of healthy volunteers and to other's observation, but we could observe significant differences in absolute values of velocity and $\Delta MV/\Delta etCO_2$ ratio during 6 min ergometer cycling between non-treated hypertensives and controls. This decreased and shorter reactivity of the arteriolar system in hypertensive patients can not be explained by the less intensive physical activity of hypertensives during the loading. Although hypertensive patients indeed performed significantly worse than controls during the test, the time course of blood pressure, heart rate and $etCO_2$ did not differ in those hypertensives and controls who could go on with the loading up till 6 minutes ($p = 0.24$, 0.40 and 0.67 for blood pressure, heart rate and $etCO_2$, respectively). Even in that one third of the hypertensive patients who were fit enough to go on with the loading until 6 minutes, the reactivity of the arteriolar system to CO_2 decreased whereas this reactivity was stable during the test period in the controls.

The hemodynamic changes during ergometer cycling may reflect the combined neurogenic, myogenic and metabolic activation of the cerebrovascular regulative system: in addition to changes in $etCO_2$, an increase in blood pressure, heart rate, cardiac output, as well as marked changes in plasma metabolite (catecholamine, glucose, lactate, etc) levels can be expected. As decreased reactivity was found in hypertensive patients despite of BP levels reaching the autoregulatory threshold, we assume that metabolic regulation and its exhaustion has the most important role in setting the vasoreactivity response during cycling. Malatino has found "the ratio of $\Delta MV/\Delta etCO_2$" useful to describe vasomotor reactivity after hyperventilation, and a drop in this parameter coincided with the plateau of flow velocity after 2 minutes loading in our study. During physical exercise the elevation of arterial CO_2 partial pressure causes vasodilatation leading to a decrease in cerebrovascular resistance that is, in turn, responsible for the elevation in MV in basal cerebral arteries. In our study this mechanism was found to be altered in clinically symptom-free hypertensives compared to controls.

Clinical trials demonstrate the importance of treatment of hypertension and risk reduction for stroke and other cardiovascular diseases. Effective strategy for the primary and secondary prevention of stroke includes treatment of hypertension. It also assumed that antihypertensive therapy may normalize impaired cerebral blood flow autoregulation in the early stages of hypertension. We could follow the majority of our recently non-treated hypertensive patients and repeat the examination 3 years later using the standard method. As far as our knowledge,

this is the first study investigating the efficacy of antihypertensive therapy on cerebral hemodynamics using the TCD combined ergometer cycling. In our study, using same and standard method, decreased ratio of $\Delta MV/\Delta etCO_2$ was found in non-treated hypertensives after 3 years. It means the antihypertensive therapy (independently of mode of action) has a beneficial effect on cerebral hemodynamics, slowing down the vessel wall elasticity changes. Although our subjects' number was small, these beneficial changes could be demonstrated by TCD combined ergometer cycling.

There are several advantages and some disadvantages of this method. Cerebral hemodynamic values at rest and after stimuli could be continuously measured, ergometer cycling is a physiological stimulation, therefore this method represents a more real-life situation than CO_2 inhalation or acetazolamide stimulation; ergometer cycling simulates the physical stress hypertensives are exposed to during their everyday activities. The relatively simple, non-invasive test could be used for repeated measurements.

Our method also has some disadvantages. There are certain patients for whom we do not consider the test suitable, e.g patients who have heart disease and patients with movement disorders. Some hypertensives do not tolerate the loading as long as the age matched healthy volunteers. Therefore a shorter duration of loading should be planned in hypertensives than in normal subjects. Interpreting test results might also be difficult as mean velocity changes in cerebral arteries during physical exercise may reflect not only metabolic, but other mechanisms as well.

6.3. Measurement of common carotid artery intima media thickness (IMT) in young hyperlipidemic patients

Intima-media thickness measured by B-mode ultrasound in the common carotid arteries is an accepted marker of atherosclerosis. The association of carotid artery IMT with the well-known cardiovascular risk factors, such as age, smoking and hypertension, has been demonstrated in several studies, and IMT was linearly related to the total number of cardiovascular risk factors. CCA IMT has been used as a surrogate quantitative endpoint in recent clinical trials on the progression of atherosclerosis. For these reasons we found it justified to use CCA IMT measured by B-mode Doppler to quantitate the atherosclerotic process in young subjects.

The role of triglyceride and cholesterol in carotid bifurcation atherosclerosis remained controversial even after large angiographic studies. There are several reports that question the role of cholesterols, triglycerides or both, in atherosclerosis, quantified by IMT. Lavrencic found whereas Ylitalo did not find greater IMT of the CCA in patients with familial hyperlipidemia than healthy subjects. The results of recent population studies are also equivocal: cholesterol was found to have a strong effect on IMT in the Muscatine and the Tromso but not in the Framingham studies. Triglyceride was reported to have an effect on IMT in both sexes in the Muscatine but only in women in the Tromso study. For these reasons we felt that the question whether cholesterol and triglyceride do or do not have an effect on IMT has not been completely settled and we assumed that analyzing a wide range of lipid values and considering confounding factors could help to resolve these conflicting conclusions.

To minimize the confounding effect of age, one of the strongest predictor of IMT as much as possible, we chose young subjects (mean: 44 years, all subjects below 55 years of age) for this study. To get a wide enough range of lipid values we selected such subjects for this study who had markedly elevated cholesterol and/or triglyceride values documented in their medical history. Including controls and patients, the range of actual serum levels were 3.93–25.03 mmol/l and 0.36–75.97 mmol/L for cholesterol and triglyceride, respectively, i.e. an over 6-fold difference between minimal and maximal values for cholesterol and an over 200-fold difference for triglyceride. We hoped that by minimizing the effect of age and maximizing the range of lipid values in this study we could increase the possibility to detect the effects of triglyceride and cholesterol on IMT.

There are some limitations of this study. First, extremely high serum triglyceride values might be associated with several pathologic conditions like pancreatitis, and toxic effects of several compounds might result in hypertriglyceridemia. Although our patients did not have clinical signs of pancreatitis, neither a toxic effect was known behind their high triglyceride values, such causes were not systematically searched in our patients. However, in the total group only 7 patients had triglyceride values over 20 mmol/L and we tried to diminish the effect of the skewed distribution of serum triglycerides by using a logarithmic transform in data analysis. The second limitation of this study is the fact that 28 of the subjects were on lipid lowering medication and a further 16 subjects were on antihypertensive therapy. It has been reported that both lipid lowering and antihypertensive treatment might result in the reduction of IMT.

In the control group none of the subjects had lipid lowering treatment, and only one had antihypertensive medication. These treatments, if they had any effect on IMT at all, would have decreased and not increased the difference in IMT between the controls and patients.

In summary, we found a positive association between IMT and total cholesterol and a negative relationship between HDL-C and IMT, whereas we could not confirm the role of triglyceride in early onset intima-media changes. Our results were consistent when an independent sonographer repeated the measurements.

6.4. Possible risks of early occlusive carotid artery disease

Examining an elderly patient group may mask the effects of risk factors of early atherosclerosis, therefore we examined the role of several potential risk factors of early atherosclerosis in young subjects (mean age 48 years). The prevalence of carotid artery stenosis is low in this age group: 0 and 0.7% of women and 0 and 1.2% of men below 50 and below 60 years of age were reported to have more than 35% stenosis in the population based Tromso Study. In our study the cutoff for age was 55 years, so the prevalence of carotid stenosis in that age group can be assumed to be below 1%. Indeed, we identified the 65 patients below 55 years of age with carotid stenosis >30% or occlusion among the over 12.000 subjects examined between September 1999 – November 2001. Although our sample was balanced for gender in the stenosis group, the majority of young patients were males in the occlusion group. Male dominance was characteristic for carotid occlusion in the Tromso study as well: all 13 patients with right sided carotid occlusion out of the sample of 6420 subjects were males.

The role of triglyceride and cholesterol in carotid bifurcation atherosclerosis remained controversial. IMT was found to correlate with LDL-C, and triglyceride and inversely with HDL-C. In the fasting state LDL cholesterol, but not HDL cholesterol and plasma triglycerides were related to IMT in 50-year-old white men. Lipoprotein (a) was also suggested as a risk factor for carotid atherosclerosis. In the present study lipoprotein (a) was the only lipid parameter that differed between patients with occlusive carotid artery disease and controls.

The role of homocysteine in atherosclerosis was suggested over 30 years ago and high-normal homocysteine concentrations were associated with an increased prevalence of carotid artery wall thickening in a younger patient group. Homocysteine exerts its effects through a mechanism involving oxidative damage, including oxidative modification of LDL. Moderate hyperhomocysteinemia predicted the severity of cerebral atherosclerosis in patients with cerebral infarction and a strong association was found between plasma homocysteine and ischemic stroke due to large artery atherosclerosis. Homocysteine was also higher in our patients with occlusive carotid artery disease, but the difference was on the margin of statistical significance.

The oxidation of LDL in the subendothelial space is one of the early events in atherogenesis. Another hypothesis emphasizes the role of inflammation. Inflammation and lipid peroxidation might have some connection in the development of early atherosclerosis, as both IMT and ΔT at V_{max} were the smallest in the lowest CRP quartile. Our finding does not exclude the role of oxidized LDL in the pathogenesis of carotid atherosclerosis. Oxidative modification of LDL take place in the artery wall under a complex set of conditions affected by plasma components and cell metabolism. Increased oxidative sensitivity of LDL is not the only determinant in oxidative modification of lipoproteins.

Atherosclerosis is an inflammatory disease; the role of infection in the disease is very controversial. Of the inflammatory indicators elevated CRP was associated with an increased intima to media thickness of the common carotid artery. The most consistent finding in our study was the increased level of inflammatory markers (WBC, CRP, fibrinogen) in the patient groups. Fibrinogen has been found to be a significant predictor of IMT by several studies. Our finding that smoking explains the difference in fibrinogen among the groups draws the attention to the fact that in such studies controlling for smoking should always be performed in data analysis. Some of the differences however remained significant among groups after controlling for smoking status, suggesting that smoking by itself cannot explain the increased levels of inflammatory markers in patients with occlusive carotid artery disease.

From our results we can conclude that oxidative resistance of LDL does not play a marked role in early onset carotid atherosclerosis – or if LDL oxidation does occur as a local early process in the carotid artery wall, it can not be assessed by the examination of isolated LDL for oxidative resistance. Therefore, measurement of the oxidative resistance of LDL can not

be recommended as a routine screening tool to identify subjects prone to early atherosclerosis. Although homocysteine had a marginally significant role, the higher levels of inflammatory markers suggest that in younger age inflammation might have a more important role in the development of early atherosclerosis than other factors including serum lipids and homocysteine. Of the modifiable risk factors smoking has been proved again to be a significant factor in early onset carotid atherosclerosis, and a factor that should be controlled for in studies on atherosclerosis.

7. Practical importance of the results

We investigated *young subjects* with major stroke risk factors, such as *hypertension*, *hyperlipidemia* and *occlusive carotid artery disease*. Using the relatively simple, non-invasive *neurosonological methods* we could detect the early stage of atherosclerosis. We could found *some factors* that differentiate young patients with carotid atherosclerosis from those without early atherosclerotic changes.

► Our data showed, that the presence of a carotid bruit is an adequate clinical predictor of extracranial carotid artery occlusive disease. The absence of a bruit on carotid auscultation can rule out stenosis in a large percentage of patients, but in patients who are suspected to have carotid stenosis, it seems inappropriate to restrict the investigation to auscultation of the neck. *Carotid auscultation is a useful, fast screening procedure, and it should remain part of the general physical examination.*

► Recently *ergometer cycling combined with continuous TCD monitoring* proved to be useful for the evaluation of cerebral hemodynamics in normal subjects. As far as our knowledge, this was the first study investigating *non treated hypertensive patients*, and the *effect of 3-year antihypertensive therapy* on cerebral hemodynamics using the the same method. We conclude that TCD registration combined with physical loading is a sensitive tool for detecting early impairment of cerebral hemodynamics in hypertensive patients. Antihypertensive therapy may have a beneficial effect on cerebral hemodynamics, slowing down the vessel wall elasticity changes.

► Increased common carotid artery IMT is an early marker of the atherosclerotic process. To examine young subjects with a *wide range of lipid values* we found a *positive association between IMT and total cholesterol* and *a negative relationship between HDL-C and IMT*, whereas we *could not confirm the role of triglyceride in early onset intima-media changes*. Our results were consistent when an independent sonographer repeated the measurements.

► We investigated patients with *carotid artery occlusive disease below 55 years of age.* We could not detect any change in oxidative resistance of LDL isolated from peripheral blood. Therefore, *measurement of the oxidative resistance of LDL can not be recommended as a routine screening tool* to identify subjects prone to early atherosclerosis. The higher levels of *inflammatory markers suggest that in younger age inflammation might have a more important role in the development of early atherosclerosis* than other factors including serum lipids and homocysteine.

8. Publications

8.1. The thesis is based on the following full papers:

1. Bereczki D, Balla G, Csiba L, Jeney V, Valikovics A, Magyar T, Balla J. A possible role of decreased oxidative resistance of low-density lipoproteins in the early formation of carotid atherosclerosis. *Medical Hypotheses* 2001;56:694-696. IF:0,745
2. Magyar MT, Valikovics A, Bereczki D, Ficzere A, Czuriga I, Csiba L. Transcranial Doppler monitoring in hypertensive patients during physical exercise. *Cerebrovasc Dis* 2001;12:186-91. IF:1,665
3. Magyar MT, Nam EM, Csiba L, Ritter MA, Ringelstein EB, Droste DW. Carotid artery auscultation-anachronism or useful screening procedure? *Neurol Res* 2002;24:705-8. IF:1,176
4. Magyar MT, Szikszai Z, Balla J, Valikovics A, Kappelmayer J, Imre S, Balla G, Jeney V, Csiba L, Bereczki D. Early onset carotid atherosclerosis is associated with increased intima-media thickness and elevated serum levels of inflammatory markers. *Stroke* 2003;34:58-63. IF:5,33
5. Magyar MT. Új módszer a hypertonia cerebralis szövődményeinek diagnosztikájában. *Orvostudományi Értesítő* 2003;76:95-99.
6. Magyar MT. A gyulladás és az atherosclerosis kapcsolata. *Metabolizmus* in press
7. Magyar MT, Paragh G, Katona E, Valikovics A, Seres I, Csiba L, Bereczki D. Serum cholesterols but not triglycerides are associated with intima-media thickness of the common carotid artery in young subjects with a wide range of lipid values. *Circulation* közlésre elküldve
8. Magyar MT, Valikovics A, Czuriga I, Csiba L. Effect of antihypertensive treatment on CO_2–reactivity of cerebral arterioles during physical exercise. *Cerebrovasc Dis* közlésre elküldve

8.2. Conference abstracts related to the thesis:

1. Magyar T, Valikovics A, Oláh L, Czuriga I, Csiba L. Changes of cerebral blood flow during physical exercise in controls. *Eur J Neurol* 1998;5(Suppl 1):S28.
2. Magyar T, Csiba L, Valikovics A, Oláh L, Czuriga I. Transcranial Doppler Investigations in controls during physical exercise. *Cerebrovasc Dis* 1998;8(Suppl 8):14.

3. Valikovics A, Magyar T, Oláh L, Czuriga I, Csiba L. Changes of cerebral blood flow during physical exercise in hypertensive patients. *Eur J Neurol* 1998;5(Suppl 1):S13.
4. Csiba L, Valikovics A, Magyar T, Oláh L, Czuriga I. Transcranial Doppler investigations on hypertensive patients during physical exercise. *Cerebrovasc Dis* 1998;8(Suppl 8):18.
5. Magyar T, Valikovics A, Fekete I, Bereczki D, Czuriga I, Csiba L. Transcranial Doppler monitoring in controls during physical exercise. *Cerebrovasc Dis* 1999;9(Suppl 2):43.
6. Valikovics A, Magyar T, Fekete I, Bereczki D, Czuriga I, Csiba L. Transcranial Doppler monitoring in hypertensive patients during physical exercise. *Cerebrovasc Dis* 1999;9(Suppl 2):37.
7. Valikovics A, Magyar T, Czuriga I, Csiba L. Transcranial Doppler monitoring during physical exercise in patients with stroke risk factors. *Cerebrovasc Dis* 2000;10(Suppl 1):31.
8. Bereczki D, Balla J, Valikovics A, Kappelmayer J, Olvasztó S, Magyar T, Fekete I, Csiba L. Degree of carotid stenosis correlates with serum homocysteine but not with cholesterol or triglyceride level in patients below 55 years of age. *Stroke* 2000;31:2792.
9. Magyar T, Valikovics A, Csiba L, Balla J, Bereczki D. Comparison of on-line and off-line measurements of intima-media thickness in patients with atherosclerotic carotid artery disease. *Cerebrovasc Dis* 2001;11(Suppl 4):52.
10. Valikovics A, Balla J, Magyar T, Kappelmayer J, Csiba L, Bereczki D. Relationship between ultrasonographic and serum lipid parameters of young patients with occlusive disease of carotid artery. *J Neurol Sci* 2001;187(Suppl 1):S22.
11. Magyar T, Valikovics A, Czuriga I, Csiba L. Effect of antihypertensive treatment on CO_2 reactivity of cerebral arterioles during physical exercise. *Cerebrovasc Dis* 2002;13(Suppl 4):24.
12. Magyar T, Paragh G, Valikovics A, Csiba L, Bereczki D. Extreme hyperlipidemia is associated with increased intima-media thickness of the common carotid artery in patients below 55 years of age. *Eur J Neurol* 2002;9(Suppl 2):107.
13. Valikovics A, Balla J, Magyar T, Szikszai Z, Imre S, Csiba L, Balla G, Kappelmayer J, Bereczki D. Serum homocysteine and C-reactive protein are higher in young patients with occlusive carotid artery disease. *Cerebrovasc Dis* 2002;13(Suppl 3):28.
14. Magyar T. Funkcionális transcraniális Doppler vizsgálatok a stroke rizikófaktoraiban. *Ideggy Szle* 2003;56:136-137.

8.3. Other publications:

1. Valikovics A, Magyar T, Fülesdi B, Rautenberg W. Színkódolt transcranialis Doppler-szonográfia- modern noninvazív eljárás a cerebrovascularis megbetegedések diagnosztikájában. *Agyérbetegségek* 1998;II:8-10.
2. Valikovics A, Magyar T, Bereczki D, Hegedüs I, Kappelmayer J, Fekete I, Fülesdi B, Csiba L. A D-dimer szint és az agyi embólia összefüggése. *Ideggy Szle* 1999;52:94-98.
3. Balla J, Blaskó G, Magyar MT, Barna E, Bereczki D. Should soluble CD40 ligand be measured from serum or plasma samples? *Arterioscler Thromb Vasc Biol* letter, in press IF:5,816

8.4. Other abstracts:

1. Valikovics A, Magyar T, Hegedüs I, Kappelmayer J, Oláh L, Bereczki D, Csiba L. Microembolisation in the middle cerebral artery of patients with high risk for embolic stroke. *Eur J Neurol* 1996;3(Suppl 5):47.
2. Fekete I, Valikovics A, Bereczki D, Magyar T, Szikszai Z, Imre S. Examination of hemorheological parameters in comparison with blood flow velocity of the middle cerebral artery in stroke patients. *J Neurol Sci* 1997;150(Suppl):S85.
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4. Csiba L, Magyar T, Valikovics A, Oláh L, Ficzere A. Haemodynamic and neuropsychological correlates in symptom-free hypertension. *Cerebrovasc Dis* 1999;9(Suppl 2):37.
5. Csiba L, Magyar T, Ficzere A, Kemény V, Valikovics A. Neuropsychological and hemodynamic consequences of essential hypertension. *Eur J Neurol* 1999;6(Suppl 3):128.
6. Tóth Á, Kisely M, Valikovics A, Magyar T, Sziklai I. The effect of vestibular stimulation on blood flow velocity in the middle cerebral artery. *Laryngo-Rhino-Otol* 2000;79:S324.
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9. Kerényi L, Molnár S, Ritter M, Magyar T, Ringelstein EB, Csiba L. Correlation of atherosclerotic changes between carotid, coronary and femoral arteries. Comparison of in vivo findings with autopsy. *Cerebrovasc Dis* 2003;16(Suppl 4):54.
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