SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Morphological and functional cerebral ultrasound studies in stroke risk factors

by Péter Siró

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF NEUROSCIENCES

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Supervisor: Béla Fülesdi MD, PhD, DSc

Doctoral School of Neurosciences, University of Debrecen

Head of the **Examination Committee**:	Miklós Antal MD, PhD, DSc
Members of the Examination Committee:	Ferenc Bari MD, PhD, DSc
	Pál Soltész MD, PhD, DSc

The Examination takes place at the Department of Anesthesiology and Intensive Therapy of Medicine, Faculty of Medicine, University of Debrecen, on June 15, 2015, at 10 a.m.

Head of the **Defense Committee**:	Miklós Antal MD, PhD, DSc
Reviewers:	Pál Barzó MD, PhD, DSc
	László Oláh MD, PhD
Members of the Defense Committee:	Ferenc Bari MD, PhD, DSc
	Pál Soltész MD, PhD, DSc

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, on June 15, 2015, at 3 p.m.

Introduction

According to epidemiological studies regarding the most important cardiovascular risk factors and the complications related to anesthesia, both hypertension and diabetes mellitus represent increased risk. The prevalence of perioperative cardiovascular complications is 6% in the average population, this prevalence is 11-12% in case of hypertension and about 9% in diabetes. In case of chronic hypertension the increased perioperative risk means the deterioration of left ventricular failure, the development of cerebrovascular disease and renal failure. In diabetic patients the 3,5-fold increased risk of neurological and 5-fold increased risk of renal complications compared to the normal population can be emphasised. From these data it is obvious that both risk factors have high rank on the list of perioperative complications. The detailed pathophysiological background of the perioperative neurological dysfunctions is not explained yet, though the early detection and adequate pre-operative treatment of the affected patients would probably decrease the perioperative morbidity and mortality ratio. As a part of our study for improving the recognition of patients at risk, we investigated the cerebral hemodynamics in different cardiovascular risk factors (diabetes and hypertension).

During cerebrovascular surgery performed on the carotid arteries there are three hemodynamically critical phases for the patient: the first one is the narcosis induction, in this period we can expect a 25-30% reduction of the systemic blood pressure, the second one is the exclusion period of the carotid endarterectomy which represent a temporary carotid occlusion, the third one occurs after the reopening of the vessel when an overflow compromises the brain adapted to hypoperfusion, the relatively high blood flow can cause hyperperfusion syndrome. Thus from the anesthesiologist's point of view these are the phases of the cerebrovascular surgery when the maintenance of optimal settings of blood pressure and oxigenisation need increased attention.

Our aim was to test whether cerebrovascular compensating mechanisms can be damaged in the presence of cerebrovascular risk factors. There's a constant requirement connected to all anesthesiology procedures to decrease the perioperative brain damage (perioperative stroke, postoperative delirium, postoperative cognitive deficit). We should define the pathophysiological processes behind the decreased cerebrovascular reactivity this way we can identify the high risk patients in the preoperative period, and then we can adapt the type of anesthesia and perioperative care to the patient's condition.

Aims

The subject of this present thesis is the observation of the potential utility of some non-invasive diagnostic procedures in the most important risk factors of stroke. We studied 3 different pathological entities, thus we can divide our aims into three issues.

1. ASSESSMENT OF CEREBROVASCULAR REACTIVITY USING BREATH HOLDING TEST IN HYPERTENSIVE ADOLESCENTS
   The aim of this work was to study arteriolar functions with a simple vasoreactivity test the breath holding test. When we began work there were no available data about the possible damage of cerebral arteriolar functions in hypertensive adolescents, so we regarded as our main purpose to study that.
2. CAROTID INTIMA-MEDIA THICKNESS AND CEREBROVASCULAR REACTIVITY IN LONG TERM 1 TYPE DIABETES MELLITUS:

   The goal of our next investigation among the less studied type1 diabetic patients was to assess the cerebrovascular reactivity and intima-media thickness with a simple method which doesn't need drug administration, cause less possible complications, and may be adequate to use in wide range of patients. We wanted to discover the possible deterioration of cerebrovascular reactivity and identify the parameters responsible for the functional and morphological damage.

3. ASSESSING THE CORRELATION BETWEEN COLLATERAL FUNCTION OF THE CIRCLE OF WILLIS AND ISCHEMIC STROKE**:**
   We designed a case-control study to determine whether the non-functioning collaterals are independent risk factors for ischemic stroke. We used a non-invasive, bed-side applicable ultrasound method combined with carotid compression test.

Patients and methods

ASSESSMENT OF CEREBROVASCULAR REACTIVITY USING BREATH HOLDING TEST IN HYPERTENSIVE ADOLESCENTS

As a part of Debrecen Hypertension Study (DHS) we investigated adolescents between the age14 and 19 years. The original goal of the DHS was to determine the point prevalence and risk factors of hypertension in the above mentioned age period in a population based study. We enrolled all the secondary school students educating in Debrecen during the time period of the study (10 359 students).

Diagnostic criteria for hypertension

For measuring blood pressure we used an Omron M4 digital, oscillometry principle based device (Omron Healthcare GmbH, Hamburg, Germany). The measurements were performed at the same time in the class rooms. We performed 3 measurements in all cases, there were 5 minutes resting periods between the measurements. We averaged the 3 results and we produced 32 subgroups according age, gender, height. We determined the 90th percentile blood pressure value in all subgroups, in the further analysis we used these values as reference value. Then just the 1641 adolescents whose blood pressure exceeded the 90th percentile had

further investigations. In these adolescents 3-3 more blood pressure measurements were performed in a 3 months periods, so we verified or excluded the diagnosis upon the average of 9 measurements. We verified the diagnosis when either the systolic or the diastolic value exceeded the 95 percentile of the population. According this definition we found 216 cases (2,01% of population) In this study randomly recruited 55 hypertonic and 35 healthy adolescent participated, we obtained informed consent all from the participants.

Transcranial Doppler measurements

Middle cerebral artery blood systolic, diastolic and mean flow velocities were measured, we used a DWL Multidop T type transcranial Doppler device. The vessels were insonated at 50-mm depth on both sides. We calculated the pulsatility index according the Gosling formulation:

$$\frac{\text{(systolic velocity-diastolic velocity)}}{\text{average velocity}}$$

During the measurements the patients were in resting supine position, the probe was placed on the temporal acoustic window. After getting baseline values, subjects were asked to take a normal breath and hold it for 30 seconds. A second transcranial Doppler measurement was performed at 30 seconds. In cases when the patient took a breath earlier than 30 seconds, the test was repeated and the time was reported every 5 seconds. All the measurements were performed by one investigator, who didn't know whether the subject was healthy or hypertensive.

Statistical analysis

Mean values and standard deviation was determined. We checked the distribution of the values with F-test. When the data had normal distribution we used the appropriate t-test, if not we used Wilcoxon test. A $p < 0,05$ was accepted as significant

2. CAROTID INTIMA-MEDIA THICKNESS AND CEREBROVASCULAR REACTIVITY IN LONG TERM 1 TYPE DIABETES MELLITUS

Thirty-three patients suffering from type 1 DM and 31 healthy controls entered the study. DM was diagnosed and regularly screened by a specialized diabetologist/internist from the diabetes outpatient clinic of the Health and Medical Science Center, University of Debrecen. The inclusion criteria for diabetic patients were no hypertension in previous history and normal blood concentration of cholesterol and triglycerides at the time of the study. Healthy controls were free from hypertension, DM, or cerebrovascular disease in previous history. The study was approved by the local medical ethics committee. Investigations were performed after written informed consent was obtained from the participants. Before performing ultrasonic investigations, the most important clinical and laboratory data related to diabetes were registered: age of the patients (in years), duration of diabetes (in years), glycosylated hemoglobin level as the marker of recent glucose control, microalbuminuria, and the presence

or absence of diabetic retinopathy and neuropathy. The glycosylated hemoglobin level was determined by liquid chromatography using DIAMAT equipment (Biorad, Hercules, CA) and was expressed in percentage. Microalbumin in the urine was assessed after a timed collection lasting for 24 hours and was determined in mg/day. Retinopathy was diagnosed in all cases by an experienced ophthalmologist. The diagnosis of diabetic neuropathy was based upon such clinical signs and symptoms as distal paresthesias, disturbance of superficial, and vibration senses and hyporeflexia.

Measurement of the carotid IMT was performed by a 7-MHz linear array probe (Hewlett-Packard Sonos 2000, Palo Alto, CA). During scanning, the probe was placed just behind the sternocleidomastoid muscle. The bifurcation of the carotid artery was found first, and measurements were performed 2 cm proximal from the bifurcation at the far wall to the sonographic probe. IMT was defined as the distance between the media- adventitia layer and lumen-intima interface and was expressed in millimeters. Three measurements were performed in each carotid artery; thereafter, IMT values were averaged, and these averaged values were used for further statistical analysis. IMT was assessed by the same investigator, who was unaware of the patients' grouping status (ie, diabetic or not).

Transcranial Doppler Measurements

Middle cerebral artery blood flow velocities were measured at resting state in supine position. The vessels were insonated at 50-mm depth on both sides. After getting baseline values, subjects were asked to take a normal breath and hold it for 30 seconds. A second transcranial Doppler measurement was performed at 30 seconds. In cases when the patient took a breath earlier than 30 seconds, the test was repeated and the time reported every 5 seconds. Thereafter, a 5-minute resting period was given to the patients to normalize the breathing pattern, and hyperventilation tests were started only by usual spontaneous breathing. During the voluntary hyperventilation test, patients were instructed to take deep breaths for 1 minute with the frequency of 25–28 per minute on average. At the end of hyperventilation period, transcranial Doppler measurements were repeated.

Calculation of cerebrovascular reactivity

Cerebrovascular reactivity (CVR) was defined as the percentage change of the mean blood flow velocity measured in the middle cerebral artery after 30 seconds of breath holding or 60 seconds of HV. We used the following formula for calculating CVR:

$$CVR = \frac{MCAVtest - MCAVrest}{MCAVrest}$$

where MCAV rest is resting mean blood flow velocity in the middle cerebral artery, MCAV test is middle cerebral artery mean blood flow velocity as measured after BH or HV respectively. Thus we obtained positive CVR values for BH and negative values for HV tests.

Means and standard deviations are reported for all values. Parameters with normal distribution were compared with the appropriate t tests. The Mann-Whitney test was used for comparing

data with non-normal distribution. Nonparametric data were analyzed by chi-square tests. Regression analysis was used for testing the relationship between IMT, cerebrovascular reactivity, and other numerical parameters such as age, du- ration of diabetes, microalbuminuria, and HgbAlC. A $p < 0.05$ was accepted as the level of significant difference.

ASSESING THE CORRELATION BETWEEN COLLATERAL FUNCTION OF THE CIRCLE OF WILLIS AND ISCHEMIC STROKE

Patients with clinical signs of cerebral infarction in the anterior circulation admitted to the Stroke Unit of the Department of Neurology of the Debrecen University Medical School were enrolled into the study. Only Caucasian patients were included. Brain infarction was defined as rapidly developing signs of focal disturbance or sometimes generalized loss of cerebral function lasting longer than 24 hours or leading to death, with no apparent cause other than that of vascular origin. The modified Rankin scale was used for assessment of stroke severity on admission. The patients underwent complete neurologic and physical evaluation including history taking to identify risk factors, lipid and coagulation profiles and imaging studies (duplex scanning of the extracranial arteries, echogardiography in selected cases and computer tomography (CT) scanning of the brain). CT scanning always proceeded TCD. Patients with an acute neurologic deficit attributable to other causes were excluded. The study was approved by the local ethical committee and the patients or the closest relatives gave informed consent.

Control group:

Atherosclerotic vascular patients with no clinical symptoms or history of cerebral ischemia serves as control patients. Only Caucasian patients were included. They were recruited from the vascular surgical outpatient clinic of the Academic Medical Center in Amsterdam, the Netherlands. They attended for evaluation of peripheral arterial disease. Control patients did not have CT or MRI investigation of the brain. Approval was obtained from the local ethical committee, and informed informed consent was obtained from each patient.

Assessment of collateral function

Before transcranial investigation all patients underwent duplex scanning of extracranial brain supplying arteries. A Hewlett-Packard Sonos 2000 duplex scanner was used for all ultrasound examinations. Stenosis of the carotid arteries was graded according to NASCET criteria. The cerebral arteries forming the circle of Willis were insonated through the temporal bone. Patients with partially suitable temporal windows through which only the anterior or posterior part of the circle of Willis could be visualised, were also included in the study.

In subjects with no ICA occlusion assessment of the collateral function of the circle of Willis requires carotid compression. Collateral cross-flow through the AcoA towards the middle cerebral artery was proven by reversal of blood flow in the precommunicating part of the ipsilateral anterior cerebral artery (A1) during carotid compression. Collateral flow from the basilar artery to the ICA territory through the PCoA was proven by a velocity enhancement in the precommunicating part of the ipsilateral posterior cerebral artery (P1) during carotid

compressions. A P1peak systolic velocity enhancement of more than 20% was used to define the PcoA as functional, this value being twice as much as can be expected from normal variation and measurement errors. In hypoplasia of the P1, the main stem of the posterior cerebral artery arises from the ICA. This is a common anomaly of the circle of Willis and may hamper posterior collateral flow. In this so called fetal posterior configuration of the circle of Willis, the PcoA can be visualize because of its large size and this enables direct velocity measurements in the PcoA. If carotid artery compression caused a velocity decrease in the PcoA instead of flow reversal, then the posterior collateral pathway was defined as nonfunctional.

Carotid compression

Compressions of the common carotid artery were applied for a maximum of 4cardiac cycles, low in the neck just proximal of the sternal head of clavicle. To minimize the risk of embolism compressions were performed only in patients with no atherosclerotic plaques in the proximal common carotid artery as judged by the B mode image of the duplex scan. To ensure the efficacy of the compression, a pulse oximeter (Eagle 3000, Marquette) that generated pulse tracing on a separate monitor was attached to the earlobe on the same side as the compressed artery. Flattering of this pulse wave indicated cessation of blood flow through the common carotid artery and thus adequate compression.

Carotid compression

In patients with unilateral ICA occlusion collateral supply from the contralateral hemisphere through the AcoA was proven if the A1 ipsilateral to the occluded ICA demonstrated reverse flow. In subjects with bilateral ICA occlusions the function of the anterior collateral pathway could not be assessed. Collateral flow from the basilar artery to the ICA was demonstrated if the mean blood flow velocity in the ipsilateral P1 was more than the mean blood flow velocity +2 standard deviations from an age- sex-matched group of atherosclerotic patients with no ICA occlusive disease or cerebral symptoms.

Secondary collateral pathways

In patients with ICA occlusion and insufficient collateral blood flow via the circle of Willis, secondary collateral blood flow via the ophthalmic artery and the leptomeningeal vessels might be present. In cases and controls with ICA occlusion we assessed this type of collateral flow by means of a 7.5MHz probe which was placed over the orbit using coupling gel, with eyelid closed. To prevent damage to the retina, the Doppler power was always reduced to the 10% of that required to penetrate the temporal bone. Retrograde flow in the ophthalmic artery indicated a functional ophthalmic collateral pathway. Collateral supply from the leptomeningeal arteries over the surface of the brain could not be assessed by TCCD.

Analysis

In both cases and controls the number of nonfunctional anterior and nonfunctional posterior collateral pathway was determined. Subjects were stratified according the presence of severe (>70% stenosis or occlusion) ICA occlusive disease. All calculations were based on the total number of successfully insonated collateral pathways in each group and not on the number patients. Odds ratios (OR) including 95% confidence intervals (CI) of nonfunctional posterior collateral pathways were calculated. To calculate the OR of a nonfunctional posterior

collateral pathway the symptomatic hemisphere of the case patients was considered the index hemisphere $\chi 2$ tests with Yates correction or Fisher's exact tests were used to compare proportions of categorical veiables. Significance was assumed at the 5% level.

Results

ASSESSMENT OF CEREBROVASCULAR REACTIVITY USING BREATH HOLDING TEST IN HYPERTENSIVE ADOLESCENTS

Altogether we examined 113 hypertonic and 58 healthy adolescents. Though the age was significant higher in the hypertonic group than in the control group the mean difference was half year, so presumably it has no clinical importance. The body mass index of the hypertonic patients was significantly higher. The sex ratio was the same in the different groups.

Comparison of the resting blood flow velocity

The resting blood flow velocity was significantly higher in the hypertensive adolescents than the healthy group. In contrary the pulsation index in rest did not differ significantly in the two groups (hypertensive: 1.03± vs. control: 1.08±0.19, p=0.17).

Comparison of absolute values of flow velocities after breath holding

The systolic, diastolic and mean velocity's absolute values did not differ significantly after the termination of the 30 seconds breath holding period. At the same time the pulsation index was higher at the hypertensive group.

Blood flow velocity percentage changes after breath holding:

The systolic velocities % changes did not differ in the hypertensive and healthy group. At the same time in the hypertensive group the mean, the diastolic velocity values and the pulsatility index % changes was significantly lower than the normal group

CAROTID INTIMA-MEDIA THICKNESS AND CEREBROVASCULAR REACTIVITY IN LONG TERM 1 TYPE DIABETES MELLITUS

The age of the diabetic patients and controls was similar (40.0 ± 6.6 years and 41.3 ± 10.4, respectively), as was the female/male ratio (19/14 and 16/15, respectively). The average duration of type 1 DM was 21.5 ± 6.9 years (range, 10–38 years). Thus, in the present study, patients suffering from DM for a long time were included. Glycosylated hemoglobin levels were 8.5 ± 1.1% on average (range, 6.5–11.2%) indicating fairly good diabetes control. Among the target organ damages, micro- albuminuria indicated a large variety of nephro-pathic severity, ranging from 2–1300 mg/day (mean ± SD: 103.0 ± 251.1 mg/day). In the majority of the patients, both diabetic retinopathy (n=25) and neuropathy (n=22) could be observed.

Intima-Media Thickness Measurements

Diabetic patients showed a significantly larger IMT (mean ± SD: 1.0 ± 0.2 mm) than healthy controls (mean ± SD: 0.6 ±0.06 mm, $p < 0.001$).

Transcranial Doppler Measurements

Resting cerebral blood flow velocities and blood flow velocities measured after breath holding (BH) and hyperventilation (HV) are summarized in Table 1. Resting cerebral blood flow velocities were higher among control subjects than those measured in diabetic patients. The differences in absolute blood flow velocities remained consistent both after BH and HV maneuver.

Cerebrovascular Reactivity

The percentage changes of the middle cerebral blood flow velocities after BH and voluntary HV maneuver are depicted in Figure 1. BH for 30 seconds induced a less-pronounced increase in mean cerebral blood flow velocity of the diabetic patients than in healthy control persons, indicating impaired vasodilatory ability of the cerebral microvasculature. When vasoconstrictor reactivity was assessed, the results showed a similar pattern: Diabetic patients reacted less intensively to HV than did nondiabetic controls

Relationship between IMT and Clinical Factors

When assessing the relationship between IMT and different clinical factors among diabetic patients, the age was the most important factor being related to severity of IMT ($r=0.53$, $p= 0.01$). In contrast, IMT was independent from duration of DM and severity of microalbuminuria. Similarly, when patients were divided into two groups based on presence or absence of neuropathy, IMT values were similar in the two group (0.93 ± 0.2 mm in the absence and 1.03 ± 0.23mm in the presence of neuropathy, respectively). Similar observation was made after assessing IMT in patients with or without retinopathy(1.02 ± 0.25 mm in patients with retinopathy vs.1.94 ± 0.06 mm in patients without retinopathy).

Relationship between Cerebrovascular Reactivity and Clinical Parameters in Diabetic Subjects

No correlation was found between IMT and percent increase of middle cerebral artery mean blood flow velocity after BH. Similarly, no relationship has been found between IMT and percent decrease of the middle cerebral artery mean blood flow velocity after voluntary HV.

ASSESSING THE CORRELATION BETWEEN COLLATERAL FUNCTION OF THE CIRCLE OF WILLIS AND ISCHEMIC STROKE

We performed TCCD in a total of 109 patients with an acute ischemic stroke in the anterior circulation. There were 78 men and 31 women with a mean age of 66 years (range 38-91 years). Of the 109 stroke patients TCCD examination was conclusive in 75 patients, 60 men and 15 women with a mean age of 64 years (range 41-91 years). Reasons of failure of TCCD and baseline characteristics of the remaining subjects are listed in table 1. The

median time between the onset of neurologic symptoms and TCCD was 5 days (range 1-85 days). Twenty-six case patients had severe ICA occlusive disease. In the 2 of the 21 cases with ICA occlusion it was present bilaterally. In 5 of the 26 patients with severe ICA occlusive disease the symptoms were related to the hemisphere contralateral to the obstructed ICA. Bilateral vertebral artery occlusions impending posterior collateral flow were not found.

It was possible to assess the function of the collateral pathway in 69 of the 75 cases (table 2). In 4 patients the arteries forming the anterior part of the circle of Willis could not be adequately insonated and in 2 patients interhemispheric cross flow through the anterior collateral pathway could not be assesed due to bilateral ICA occlusion. In 8 of the 150 hemispheres of the 75 cases, the posterior collateral pathway could not be assesed due to suboptimal insonation conditions..

Controls

The control group consisted of 113 atherosclerotic vascular disease patients, 79 men and 34 women with a mean age of 62 years (range 35-89 years). The difference in mean age between the cases and controls was statistically significant (p=0,008).Off the 113 control patients, TCCD was conclusive in 100 patients, 75 men and 25 women, with the mean age of 61 years (range 35-89 years). Nineteen control patients had severe ICA occlusive disease. Two patients had bilateral ≥70% ICA stenosis, and 8 showed a unilateral ICA occlusion of which 2 had a contralateral ≥70% ICA stenosis as well. Bilateral vertebral artery occlusions impending posterior collateral flow were not found. The function of the anterior collateral pathway could be adequately assessed in 99 control patients. It was possible to assess the function of the posterior collateral pathway i n 193 of the 200 hemispheres.

Collateral function

A nonfunctional anterior collateral pathway in the circle of Willis was found in 33% of the cases and in 6% of the controls (p<0,001). The posterior collateral pathway was nonfunctional in 57% of the cases and in 43% of the controls (p=0,002). Figure 2 shows stratification of cases and controls with severe ICA occlusive disease. In cases and controls with severe ICA occlusive disease, the anterior collateral pathway was nonfunctional in 48 and 11%, respectively (p=0,03). The ipsilateral posterior collateral pathway was nonfunctional in 60 and 33% (p=0,13). In cases and controls without severe ICA occlusiv disease, the anterior collateral pathway was nonfunctional in 26 and 5% respectively (p=0,02). The posterior collateral pathway was nonfunctional in 58 and 45% (p=0,19). A higher number of nonfunctional anterior collateral pathways was found in stroke patients with severe ICA occlusive disease than in stroke patients without severe ICA occlusive disease, 48 versus 26%, respectively; however, the difference was not statistically significant (p=0,12). The difference in the number of nonfunctional anterior and posterior collateral pathways between control patients with a without severe ICA occlusive disease was not significant either. Neither adjustment for time between stroke onset and TCCD adjustment nor adjustment for age and sex significantly influenced the frequency of nonfunctional collaterals. In table 3, the prevalence and OR of nonfunctional anterior and nonfunctional posterior collateral pathways in patients with severe ICA occlusive disease

are shown. In this group a strong association between a nonfunctional anterior collateral pathway and ischemic stroke was found (OR=7,33, 95% CI= 0,77-12,04).

Stroke Characteristics

Of the 26 stroke patients with severe ICA occlusive disease, only 2 showed a watershed infarct on CT. In 17 patients territorial infarct was found. In 2 of these patients the lacunar infarcts were classified as old lesions. In 2 patients no lesion was found. Of the 49 stroke patients with no severe ICA occlusive disease 25 showed territorial infarcts, 4 showed a combination of territorial and lacunar infarcts and 10 showed lacunar infarcts. In 3 of these patients the lacunar infarcts were classified as old lesions. In 10 patients no lesion was found. In the 15 patients with lacunar infarcts on CT, the anterior and posterior collateral pathways were nonfunctional in 27 and 61% respectively, compared with 35 and 56% in the 60 patients with nonlacunar infarcts. These differences were not statistically significant. When patients with severe carotid artery disease and nonlacunar infarcts were compared to patients with no severe carotid artery disease and lacunar infarcts, the anterior and posterior collateral pathways were nonfunctional in 50 and 55% and 25 and 44% respectively, which was again not significant probably due to small numbers.

Of the 75 cases, 56 underwent echocardiography. Of these 56 cases, 19 had severe ICA occlusive disease and 37 had no severe ICA occlusive disease. A potential cardiac source of emboli was found in 9 (47%) and 18 (49%) patients respectively. In 75% of the patients with severe ICA occlusive disease but no cardiac source of emboli, the anterior collateral pathway was found to be nonfunctional as compared with 22% in those with severe ICA occlusive disease and with a cardiac source of emboli (p=0,004). For the posterior collateral pathway no significant differences were found between groups with or without cardiac emboli.

Discussion

ASSESSMENT OF CEREBROVASCULAR REACTIVITY USING BREATH HOLDING TEST IN HYPERTENSIVE ADOLESCENTS

In our study we could demonstrate that the resting blood flow velocity in the ACM is significantly higher in the hypertensive adolescents than the normotensive group. Furthermore we demonstrated that the cerebral vasoreactivity is decreased in the hypertensive adolescents.

The resting blood flow velocity values we measured are in the same range with the reference values published by Brouwers et al. All in the resting velocity values (systolic, diastolic and mean) there were a significant difference between the two groups. Between the pulsatility indices there were no significant difference. The results of previous studies regarding the rest flow velocities are contradictory: while some of the authors found similar velocities in hypertensive and healthy subjects, others like us demonstrated higher blood flow velocities in hypertensive patients. The blood flow velocity deacreases by age, Lipsitz et al. compared blood flow velocity in healthy young and elder persons and elder hypertensive patients, they found while the younger healthy subject's blood flow velocity were higher than both the healthy, both the hypertensive group, the two elder groups did not differed significantly. Troisi et al. had a similar observation. Previous studies about

hypertensive patients' cerebral hemodynamics investigated mainly the older population. Presumably the observed slight difference in younger age between hypertensive and healthy persons diminish by increasing age.

Previously, different investigators observed decreased cerebral vasoreactivity to hypo- and hypercapnic stimuli in hypertensive patients compared to healthy persons. During breath holding hypercapnia develops. We observed decreased vasoreactivity to hypercapnia in hypertensive adolescents comparing to healthy adolescents. It is well-known that hypetension modifies the cerebral microvascular functions (vasodilatation and vasoconstriction). The increase of the resting tone of the cerebral vessels and the decrease of the endothelin dependent relaxation of the cerebral arterioles is in the background of this process. According the remodeling theory the chronic elevated blood pressure causes the increase of the wall-lumen ratio and this way it deteriorates the constrictive and dilative ability of the vessels. It is not clarified yet, how long time period is needed to hypertension to develop this alterations in the cerebral arteries. It's an undoubted fact that when hypertensive patients receive adequate treatment the altered vasoreactivity get back to normal 3 months after the initiation of the therapy. So it's supposed that the adaption of the cerebral arterioles tone to the elevated blood pressure develop within weeks, possibly within months. Since in most of the cases we diagnosed hypertension in this present study we have no sufficient data about the duration of the hypertension or the efficacy of the potential treatment.

In a few sentence we should mention the limitations of our study. It is well documented in the literature that the variability of vasoreactivity produced by BH test is relatively high, our observations confirm this fact as well. We determined the length of the BH period 30 secundum, because though this period is long enough to provide the critical CO2 level elevation, it is short enough to be tolerable for the patients. The results of this present study primarily are able to contribute to the investigation of pathophysiology, the sensibility of the method is not adequate to identify the pathology of single cases, so we can appreciate this is a limitation of our study as well.

Closing remarks: we demonstrated that in hypertensive adolescents the cerebrovascular reactivity reduced comparing to healthy adolescents. Important conclusion of our study that the decreased function of the cerebral arteries can be detected in this early period, when apparent cardiac or cerebrovascular symptoms are missing. Further prospective studies can determine the potential clinical relevancy of this altered cerebrovascular reactivity. Elaboration of population based screening investigations, the adequate treatment and follow up of the high risk patients is essential to the prevention of the cardiac and cerebrovascular complications of hypertension.

CAROTID INTIMA-MEDIA THICKNESS AND CEREBROVASCULAR REACTIVITY IN LONG TERM 1 TYPE DIABETES MELLITUS

In the present study, we demonstrated an increased IMT in patients suffering from type 1 DM. Additionally, we found a decreased cerebrovascular reactivity to both vasodilatative and vasocontrictory stimuli in diabetic subjects. To our knowledge, this is the first study assessing the effect of hyper- and hypocapnia on the reactivity of the cerebral vasculature in type 1 DM patients. Affection of the large- and medium-size vessels is a well-known phenomenon in DM, resulting in a higher incidence of carotid, coronary, and peripheral

arterial atherosclerosis. IMT in the common carotid artery has been demonstrated to be an excellent indicator for early atherosclerosis, and very recently it has been introduced as a marker of cardio- and cerebrovascular risk assessment. In diabetic patients, IMT was associated with higher levels of microalbuminuria and retinopathy, indicating that vasculopathies in the different vascular beds develop in a parallel fashion. In our study, we were able to demonstrate a significant positive relationship between the age of the diabetic subjects and IMT, but IMT was not related to duration of diabetes. It has to be noted that all our patients suffered from DM already for a long time (the average duration of the disease was 21.6 years) and the majority of subjects have had diabetes for more than 10 years. Therefore, the proportion of non-retinopathic patients (n=7) was too small to demonstrate significant differences between IMTs of the retinopathy and non-retinopathy groups because the majority of the patients already showed clinical signs of retinopathy. Baseline cerebral blood flow velocities were significantly lower in diabetic patients compared with healthy control subjects. Basically, there may be two possible mechanisms for explanation of these findings: a decreased cerebral blood flow velocity in the middle cerebral artery (MCA) may be decreased if the vessel is dilated or if the resistance vessels of the corresponding vascular territory are constricted. Peripheral vasoconstriction of the resistance vessels (eg, cerebral arterioles) as a possible cause of the decreased baseline blood flow velocities in the MCAs among diabetic patients can be well explained by the increased pulsatility indices compared with those of healthy control subjects. Higher pulsatility indices in diabetic patients have already been demonstrated by others, and they have been found to be concomitant with other microvascular complications of the disease. It has to be noted, however, that pulsatility indices do not depend only on peripheral vascular resistance; inductance, compliance and cardiac output also influence pulsatility indices. Thus, decreased cerebral blood flow velocities along with increased pulsatility indices in diabetic patients may refer to a more constricted state of the cerebral resistance vessels compared with nondiabetic subjects. The physiologic basis of BH and HV tests were discussed earlier in detail. Briefly, holding breath for 30 seconds results in an increase of extracellular pCO2 and blood flow consequently increases due to dilation of cerebral arterioles. In contrast, voluntary HV causes an increase in extracellular pO2, which is accompanied by decrease in pCO2. Both of these physiologic changes result in constriction of the cerebral resistance vessels, leading to decrease of cerebral blood flow. The theoretical basis of using BH and HV is that some dis- eases may affect arteriolar function or structure, and in those cases, these vessels have reduced ability to react to vasodilatory or vasoconstrictor stimuli. Authors of published studies have looked for vasodilatory responses of the cerebral circulation in DM, but none of the previous works assessed the function of the cerebral arterioles under vasoconstrictor stimuli. In our study, we used both vasodilatory and vasoconstrictor stimuli for measuring cerebral vasoreactivity. Although the BH test was used for testing cerebrovascular reactivity, only type 2 diabetic patients were tested.

Similar to our finding in the present study, impairment of cerebrovascular reactivity of the brain arterioles to various stimuli has been documented in DM. It has also been demonstrated that impairment in cerebrovascular reactivity correlates with duration of the disease and develops parallel with other vascular complications. In the present study, we also demonstrated an increased baseline pulsatility indices and a reduced reactivity of the cerebral arterioles against both vasodilatory and vasoconstrictor stimuli in diabetic patients. A higher activity of vasoactive substances has been described in earlier experiments in DM, which gives a good explanation for the increased arteriolar tone

observed in diabetic patients at baseline measurements. The most important observation of our study is that cerebral arteriolar function is affected in both directions (vasoconstriction and vasodilation) of the physiologic regulation of vessel tone. In our opinion, this finding refers mainly to morphologic rather than functional disturbance of the vascular regulation in DM. This finding is also supported by the pathohistological observation of cerebral small vessels of patients suffering from DM: microatheroma formation, lipid and hyaline deposits, and thickening of the basal membrane have been described.

Finally, there are limitations to our study. First, transcranial Doppler does not measure cerebral blood flow, but cerebral blood flow velocity, the changes of which are not equal but, rather, only proportional to changes of cerebral blood flow. Second, it has to be noted that results of cerebrovascular reactivity testing may be influenced by left ventricular hypertrophy as a possible consequence of DM. Because we did not assess left ventricular morphology in our sub- jects, the possible influence of left ventricular hypertrophy in diabetic subjects cannot be excluded.

To conclude, IMT is increased in patients suffering from type 1 DM compared with healthy subjects. Furthermore, we have shown an impaired response of the cerebral arterioles to hypo- and hypercapnic stimuli in IDDM subjects. Our results refer to early macro- and microangiopathic involvement of the cerebral vessels, which develop in parallel with such angiopathies of the other organs as nephropathy and retinopathy. Further prospective studies are needed to clarify the role of these macro- and microangiopathic changes in the development of cerebrovascular complications in patients suffering from DM

ASSESING THE CORRELATION BETWEEN COLLATERAL FUNCTION OF THE CIRCLE OF WILLIS AND ISCHEMIC STROKE

In this study we observed a significantly higher overall proportion of failing anterior and posterior collateral pathways in the circle of Willis of patients with ischemic stroke in the anterior circulation compared with atherosclerotic vascular disease patients with no symptoms of cerebral ischemia. In patients with severe ICA occlusive disease we found a strong association (OR=7,33, 95%CI=1,19-76,52) between a nonfunctional anterior collateral pathway and ischemic stroke. The association between nonfunctional posterior collateral pathway and ischemic stroke was less strong (OR=3,00 95%CI=0,77-12,04). In 75% of the patients with severe ICA occlusive disease and no evidence of a cardiac source of emboli, the anterior collateral pathway was nonfunctional as compared with 22% of those with a potential cardiac source of emboli (p=0,04). Tis finding underlines the vital importance of adequate collaterization in patients with severe carotid artery disease as predominant risk factor for stroke. Furthermore, these results support the hypothesis of Caplan and Hennerici that poor collaterization and marginal blood follow diminish the ability of the circulation to clear thromboemboli that are generated as well limit the available blood flow to regions rendered ischemic by embolization.

Altough the watershed infarct is considered the typical low-flow infarct caused by hemodynamic insufficiency, this type of infarct was found in 2 patients only. Both patients lacked collateral flow via the anterior as well as the posterior collateral pathway. The majoritiy of stroke patients with severe ICA occlusive disease and insufficient collateral

flow showed territorial infarcts on the CT scan, which are considered to be caused by cardiac embolism or large vessel atherothrombosis. We did not try to distinguish embolic from hemodynamic disorders, since we know from the study of Hennerici et al. that stroke mechanism cannot adequately be inferred from interpretation of stroke patterns on brain scans. However small numbers in both groups hamper solid conclusions. The number of nonfunctional anterior collateral pathways was also much higher in the case group, when cases and controls, when cases and controls without severe ICA occlusive disease were compared 26 versus 5% (p=0,002). One explanation might be that the higher rate of nonfunctional collaterals in stroke patients without hemodynamically significant ICA stenosis is not only the result of congenital anomalies, but also an expression of the severity of the general atherosclerotic damage to the vasculature of the brain. Another explanation might be that the prevalence of hypertension, which is associated with poor collateral development, was significantly higher in case group (p<0,001).

The association of circle of Willis dysfunction with the risk of ischemic stroke originates from autopsy studies performed in the 1960s. Alpers and Berry who compared the configuration of the circle of Willis of 350 normal brains with that of 194 brains with sign of infarction were among the first report that infarcted brains showed a higher proportion of hypoplastic circle of Willis collaterals. Later, in a similar study Battacharji et al. reported that a significant association between cerebral infarction and hypoplastic collaterals was demonstrated only in a group with stenosis of the carotid and/or vertebral arteries. More recently, Herdera et al. found that symptomatic patients with Y75% ICA stenosis or occlusion had fewer functional collaterals than comparable asymptomatic patients. Furthermore, Silvestrini et al. demonstrated a negative correlation between stroke severity and the number of functional collaterals in patients with ICA occlusion. Very recently, the investigators of NASCET group have shown that the presence of functional circle of Willis collaterals in patients with ≥70% ICA stenosis is associated with lower risk of hemispheric stroke and transient ischemic attack, both in the long term and perioperatively. None of these studies have analyzed the individual contributions to the results made by the anterior and posterior collateral pathways. However, our results indicate that failure of the anterior collateral pathway in particular associated with ischemic stroke. This finding strongly supports the hypothesis that the AcoA is the most important collateral artery of the circle of Willis.

The striking difference in successful insonation of the temporal window between the cases and the controls was probably caused by the age difference between the two groups, 66 versus 62 years (p=0,008). It is known that the ability to penetrate the temporal window worsens with the increasing age, especially in elderly women. The number of subjects ≥75 years of age was significantly higher in the case group as compared with the controls (p=0,02), and the mean age of females in the case group was also significantly higher,69 versus 63 years (p=0,001). Furthermore TCCD examination was particulary difficult in severely diseased often non-cooperating stroke patients, which sometimes seriously hampered the detection of the small intracranial arteries. Application of echo contrast-enhancing agent can significantly improve the detection of intracranial collaterals in patients with poor temporal window.

In summary, we found that patients suffering from an acute ischemic stroke in the anterior circulation have significantly fewer functional intracranial collaterals as compared with atherosclerotic controls with no cerebrovascular symptoms. In patients with severe ICA occlusive disease a nonfunctional anterior collateral pathway is strongly associated with

ischemic stroke. A longitudinal study of cohorts at risk might finally elucidate the relationship between carotid artery occlusive disease, the anatomical configuration of the circle of Willis, the quality of intracranial collateral flow and the risk of ischemic stroke. In ongoing or future studies investigating the prognosis of carotid artery occlusive disease the collateral ability of the circle of Willis should be assessed as it is an important determinant of patient outcome.

Summary

In the present thesis we assessed the impact of some selected stroke risk factors (hypertension, diabetes mellitus and functional ability of the willisian collaterals) on the morphological and functional changes of intracranial vessels. We have used a non-invasive method, the transcranial Doppler for this purpose, combined with different stimuly used for the testing cerebral vasoreactivity, such us breath holding test, hyperventilation test and carotid compression test. Our observations were as follows:

1. We have demonstrated for the first time in the literature that cerebrovascular reactivity after administration of the breath holding stimulus is impaired in hypertensive adolescents as compared to healthy controls.
2. We demonstrated that patients with type-1 diabetes mellitus, suffering from the disease for more than 10 years have higher intima-media thickness than non-diabetic subjects.
3. We were the first in the literature using breath holding and hyperventilations tests in patients suffering from type-1 diabetes mellitus. We demonstrated that these patients have impaired cerebrovascular reacticvtiy to both vasoconstrictor and vasodilatory stimuli.
4. We were the first using TCCD carotid compression tests for assessing the collateral patency of the circle of Willis in stroke patiens. We have shown that inappropriate anterior collaterals is more frequent in the ischemic stroke patients comparing to the atherosclerotic control group indepedently to the stroke type. In case of carotid occlusive disease the difference is maximal in this case the frequency of nonfunctional anterior collateral is 7,3 times to controls, whereas in case of non-functional posterior collaterals the frequency is 3 times.

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List of publications related to the dissertation

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