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

The importance of the intracranial heamodinamics and the morphology of the circle of Willis in the surgery of the cariotid artery

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF NEUROSCIENCES
Debrecen, 2014

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The Examination takes place at the library of the Department of Anesthesiology and Intensive Care, Faculty of Medicine, University of Debrecen at 11 a.m. 25th March, 2014.

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1. Introduction

In clinical practice the technique used during carotid endarteriectomies can be divided into two groups: in some centres the operation is done by continuous cross-clamping of the affected carotid artery and in other centres they apply a shunt during the procedure. If they use the method of continuous cross-clamping of the carotid artery stroke of hemodynamic origin is more frequent if the collateral capacity is insufficient. In this case the anterior and posterior communicating artery with reduced function is not capable of compensating the continuous cross-clamping of the carotid artery on one side. These patients are candidates for shunt protection during the procedure. In certain cases the collateral capacity may be perfect while the blood pressure is normal but in case of low blood pressure there is a major decrease in the blood flow on the side of the operation.

This fact underlines the importance of a well managed anaesthesia since most of the medications used during general anaesthesia decrease the blood pressure and cause cerebral hypo-perfusion. Two factors that can be influenced and should be paid attention to is: judging the necessity of shunt protection and managing the anaesthesia with attention specially watching out for the change in blood pressure during the operation.

In the past years different imaging and functional examinations have been introduced to judge the blood flow in the circle of Willis, since purely based on morphological findings it cannot be surely determined whether there will be a need for intaoperative shunt implant because of hemodynamic changes during the operation. Lately attention has been focused on methods where both morphological and functional information is available and may be capable of forecasting the intraoperative happenings. The present thesis summarizes the method that focuses on the collateral capacity with pathomorphological, functional and mathematical simulation modelling.

2. Arising questions and aim of the study:

a. *The morphological analysis of the circles of Willis in a non-stroke population*

We studied the structure of the circles and measured the diameters and length of the different segments. We tried to find out how many percent of typical (classic) configuration of the circle of Willis can be found in the population who did not die as a consequence of stroke and how frequent are the different variations and incomplete circles in the Hungarian population.

b. *The application of a mathematical circulation model in assessing the functional ability of the collaterals of the circle of Willis.*

We classified the function of the collaterals based on praemortem performed colour-coded transcranial Doppler examinations. With the help of postmortem examinations and the usage of a mathematical circulation model we tried to find out how does the diameter of the anterior and posterior communicating arteries effect the function of the collateral capacity and under what diameter does the collateral capacity narrow down considerably.

c. *Cerebral vasoreactivity tests in cases of occlusion and different grades of stenosis of the carotid artery.*

In our third study we wanted to find out whether the vasodilatation capacity of the cerebral arteries is different in case of symptomatic and symptom free patients with hemodynamically significant carotid stenosis and occlusion.

3. Patients and methods

The whole series of examinations consisted of 3 parts:

- The study of the morphology of the circle of Willis in patients who did not die as a result of stroke.
- The praemortem examination of the collateral capacity of the circle of Willis in patient who died from stroke and the application of the results on a mathematical circulation model.
- The application of a Doppler-acetazolamide test in patients suffering from severe carotid stenosis and occlusion.

Since we used different methology in the different parts of the examinations we describe the methods in the following order.

a. *The examination of the Willis circle morphology of patients who did not die because of stroke*

We removed the circle of Willis during the routine autopsy of the deceased patients of the Medical and Health Science Centre University of Debrecen – after the consent of the ethical committee- with the help of a pair of Cooper scissors and forceps we removed the circles from the back towards the front. The circle of Willis was dissected out after removal of the brain from the cranial cavity. All minute branches arising from the main vessels were carefully cut away. The specimen was placed into perfectly closing container and we made a proper documentation of the data of the deceased patient. We did not use any preserving chemicals and the arteries were measured within one hour. We washed the specimens and all the unnecessary connective tissue was removed. We documented every detail of the circle of Willis, all the anatomic variations and possible malformations. We than placed the circle between two glass plates and the glasses were clamped with the circle of Willis in-between the plates of glasses. Sufficient force was applied to induce complete obliteration of the vessel lumina but not to flatten the arterial walls. We measured the lengths and diameters of the arteries of the circle of Willis. After the arteries had been straightened out, the lengths of the segments were measured with a millimetre scale under a light microscope.
These measurements were measured with an approximation of 0.1 mm.

Measurements were taken at the proximal, middle and distal site of each artery segments, three half-diameters measured on each artery. This way, we documented eight length and forty-two half diameters from each Willis-circle.

Determination of the diameters of the arteries
The narrowest part of each artery was used for further analysis, because we considered that the width at this part determined the collateral ability.

Assuming the arteries to be circular, their external diameter could be calculated by the formula diameter=circumference/π. We compared the two sides both in terms of length and diameters (in case of artery segments that are in pairs) and we compared the data of the two sexes as well.

b. *Praemortem examination of the collateral capacity of patients who died because of stroke*

and the application of the results on a lineal fluid model based on mathematics

The examinations were approved by the local ethical committee and were performed at the Intensive Care Unit of the Neurological Department of the Medical and Health Science Centre University of Debrecen. The line of examinations was a part of an international project and the other project members were the work-teams of the Vascular Surgical Department, Academic Medical Centre of Amsterdam and INSEM Neurology Institute Toulouse. The patients involved in the study were moribound patients treated at the ICU of the Neurology Department because of stroke. The closest relatives of the patients consented to the examinations.

For all ultrasound examinations, a Hewlett Packard SONOS 2000 duplex scanner was used. Duplex scanning of the extracranial brain supplying carotid artery (10-MHz transducer) preceded the transcranial investigation. TCCD was performed with a 2.0 MHz transducer. Examination of the main trunk of the middle cerebral artery (M1), the precommunicating part of the anterior cerebral artery (A1), and the precommunicating part of the posterior cerebral artery (P1) through the temporal bone window was performed in a standard manner, details of which are reported in previous studies. In cases of unilateral window failure, examination of the A1 and P1 through the opposite temporal bone window was attempted.

For reliable assessment of AcoA and PcoA functional patency in patients with no ICA occlusive disease, CCA compressions are needed. Collateral supply through the AcoA to the

M1 was demonstrated by reversal of blood flow in the A1 ipsilateral to the compressed CCA, combined with an enhanced blood flow velocity in the contralateral A1 segment. Both A1 segments were routinely investigated with ipsilateral and contralateral CCA compression. Functional patency of the PcoA was defined by a peak systolic velocity (PSV) increase of >20% in the P1 ipsilateral to the compressed CCA, this value being twice as much as expected from normal variation and measurement error . The PSV increase was always measured over the highest peaks on the Doppler spectrum. If the PSV increase in the P1 was <20%, the PcoA was defined as hypofunctional. Velocity measurements were taken proximally in the A1 and P1 with the sample volume set as narrow as possible. Measurements in the P1 were taken as close as possible to the top of the basilar artery.

Compression tests. To avoid a systemic cardiovascular reaction, compressions of the CCA were applied low in the neck just proximal to the sternal head of the clavicle for a maximum of 4 cardiac cycles. To minimize the risk of embolus, compressions were performed only in patients with no atherosclerotic plaques in the proximal CCA as judged by the B-mode image of the duplex scan. To ensure the efficacy of the compression, a pulse-oximeter (Eagle 3000, Marquette), which generated pulse tracings on a separate monitor, was attached to the earlobe on the same side as the compressed artery. Flattening of this pulse wave indicated cessation of blood flow through the CCA and thus an adequate compression.

Postmortem examinations. Autopsy and measurement of vessel diameters. After the death of the patients the circle of Willis was dissected out after removal of the brain from the cranial cavity. All minute branches arising from the main vessels were carefully cut away. Pathology of the cerebral vessels was judged. The length and diameters were determined as described earlier. The morphometric data of the Willisian arteries were entered into a linear mathematical fluid model.

Computer simulation of the cerebral hemodynamic status and application of a lineal circulation model

 For each subject, the mean arterial blood pressure at the time of the TCCD examination and the morphometric data of the Willisian arteries were entered into a linear, fluid model. With the help of the model the blood pressure and blood flow values could be calculated at any part or segment of the circle.

The model reproduces the classical configuration of the circle of Willis and includes three parts: a) an afferent part (internal carotid arteries, vertebral arteries and basilar artery), b) a Willisian part (A1 segments of the anterior cerebral arteries, ACoA, P1 segments of the posterior cerebral arteries and PCoAs) and c) an efferent part (A2 segments of the anterior cerebral arteries, P2 segments of the posterior cerebral arteries and the middle cerebral arteries). For the afferent and Willisian parts, both diameters and lengths were used for calculation. For the efferent part, we used the vessel diameters and three lumped values of cerebrovascular resistance, one for each pair of cerebral arteries. After entering the data, the model calculated cerebral blood flow (in ml/s) and mean blood pressure (in mm/ Hg) in all parts of the network, including the blood pressure at the site of each bifurcation. It was thus possible to calculate the pressure drop between the two ends of an arterial segment (such as the anterior and posterior communicating arteries). In this study, we focused our interest on the blood flow through the communicating arteries and on the main parameters, which, according to the Poiseuille law, determine this flow, i.e. the diameter and length of the vessel and the pressure drop between its two ends. This method makes it possible to model what would happen to the circulation in the different segments of the circle of Willis in case of a blood pressure drop.

Statistical evaluations

Medians and ranges were reported for all values. Bilateral compression of the carotid arteries was possible in 9 cases. In these patients computer-based blood flow and perfusion pressure simulation were performed separately for both compressions. This means that in these 9 patients, two sets of blood flow calculations in each case were available and were taken into account during statistical analysis. Based on the TCCD the communicating arteries were classified as functional and non- functional. We compared diameters, calculated blood flows and pressure differences between the two ends of the communicating arteries between the functional and non-functional groups. Data of non-functional and functional communicating arteries were compared using non-parametric tests (Wilcoxon signed rank). Differences were considered to be significant in the case the probability (p) was <0.05.

c. . *Transcranial Doppler acetazolamide-test in patients with severe carotid stenosis and occlusion*

Patients were recruited from the Department of Neurology and Neurosurgery of the University of Debrecen Health and Science Center, and from the Department of Vascular Surgery, Academic Medical Center Amsterdam. Sixty-two patients (42 males and 20 females, mean age: 63.2 +/- 9.6 years, ranges: 42–81 years) suffering from hemodynamically significant stenosis or occlusion of the internal cerebral artery were entered in the study. There were 16 asymptomatic occlusions, 15 symptomatic occlusions, 16 asymptomatic stenoses, and 15 symptomatic stenoses. The distribution of the symptomatic patients according to their stroke subtypes was as follows: in the occlusion group there were 4 TIAs, 1 RIND, 1 minor stroke, 4 progressing, and 4 completed strokes, while in the stenotic group these numbers were 4-2-7-1-0, respectively. The frequency of TIAs, RIND, and progressing strokes did not differ between the two groups, while in the symptomatic occlusion group minor strokes were less frequent (p=0.0132, chi^2 test) and more completed strokes were observed

(p=0.0131, chi^2 test). Asymptomatic carotid stenoses and occlusions were occasionally found through regular ultrasound screening of the carotid arteries before operations for peripheral arterial diseases or open-heart surgery. A patient suffering from carotid artery stenosis or occlusion was considered to be asymptomatic, if there was no amaurosis fugax or neurological symptoms in the previous medical history present and brain computed tomography (CT) scan showed a normal result. Patients with symptomatic stenosis and occlusion were inpatients in the Department of Neurology, University of Debrecen, treated in the Stroke Unit; in these patients either a transient or a permanent neurological deficit could be observed and/or the brain CT showed infarctions. Carotid stenoses and occlusions were diagnosed by the 10 MHz probe of the Hewlett-Packard Sonos 2000 (USA) equipment. Both longitudinal and transverse images were performed. For measuring the severity of the stenosis on the B-mode image, the NASCET criteria were used. After assessing the carotid arteries, transcranial Doppler measurement of the middle cerebral artery was performed on both sides using EME TC 64 B transcranial Doppler sonograph. The vessel was insonated at 50 mm depth. Resting cerebral blood flow velocity measurements were followed by an i.v. administration of 1g of acetazolamide (Diamox, Lederle Parenterals, Puerto Rico). Blood flow velocity measurements were repeated at 10 and 15 minutes after

administration of this vasodilatory stimulus. Systolic, diastolic, and mean blood flow velocities, as well as pulsatility indices were registered in every case. For statistical evaluation only mean blood flow velocities were taken into account. Cerebrovascular reactivity was calculated as the percent increase of the mean blood flow velocity after administration of the vasodilatory stimulus according to the following formula:

$$CVRC= 100x\ (MCAV_{AZ}- MCAV_{REST}) / MCAV_{REST}$$

where $MCAV_{AZ}$ indicates middle cerebral artery mean blood flow velocity after administration of acetazolamide and $MCAV_{REST}$ indicates the resting value of the middle cerebral artery mean blood flow velocity. Similarly, cerebrovascular reserve capacity (CRC) was defined as the maximal percent increaseof the blood flow velocity after administratering the vasodilatory stimulus and was calculated as follows:

$$CRC= 100x\ (MCAV_{AZMAX}- MCAV_{REST}) / MCAV_{REST}$$

where $MCAV_{AZMAX}$ indicates the maximal increase of the mean blood flow velocity after acetazolamide. The asymmetry index of the cerebrovascular reserve capacity was calculated by dividing CRC on the affected side by CRC on the non-affected side.

Statistical evaluation: Means and standard deviations are reported for all values. Parameters with normal distribution were compared with the appropriate t tests. In case of multiple comparisons, Bonferroni corrections were performed. For nonparametric analysis X^2 test was used. A p value of < 0.05 was accepted as statistically significant. Statistica99 version 5.5 for Windows (StatSoft, Tulsa,Oklahoma) was used for data analysis.

4. Results

a. *Morphological analysis of the circle of Willis in a non-stroke population*

Anatomic malformations

From the removed 110 circles of Willis we found incomplete circle in 25 cases, where one or more arterial segment was missing or due to the abnormal anatomy the circle was not completely closed. The distribution of the malformations was the followings:

- In eleven case (10%) the left posterior communicating artery was missing.
- In eight case (7,27%) the right posterior communicating artery was missing.
- In four case (3,63%) we found that both posterior communicating arteries were missing.
- In one case (0,9%) the left communicating artery ran in a lateral direction without closing the circle.
- Apart from this we considered the circle incomplete in one case (0,9%) when we found a foetal configuration where the right posterior cerebral artery originated from the internal carotid artery and the first segment only existed as a connective tissue without a lumen.

In the above mentioned case the circle cannot fulfil its function as a collateral network, there is no connection between the vertebrobasilar and the internal carotid system. During our study we found incomplete circles in 25 cases (22,7%) among the circle of Willis of the non-stroke population.

Anatomic variations

We classified those circles into this category where the circles were complete but there were segment duplications or abnormal origins of the artery segments.

The distribution of the anatomic variations was the following:

- In seven cases (6,36%) we found anterior communicating artery duplication.
- In one case (0,9%) we found a duplicated first segment of the right anterior cerebral artery where the duplicated segment runs round the junction of the anterior communicating artery.
- In one case (0,9%) we found a fork like bifurcation of the anterior communicating artery.
- In one case (0,9%), we found that the anterior cerebral artery originated from a common trunk and this trunk also acted as an anterior communicating artery
- In one case (0,9%) the length of the left posterior communicating artery was actually zero and the left posterior cerebral artery run all the way to the internal carotid artery.
- In one case (0,9%) we found that both of the posterior communicating arteries originated from the left posterior cerebral artery.

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- In one case (0,9%) we found the duplication of the first segment of the left posterior cerebral artery.
- And finally in one case (0,9%) we found that the right posterior communicating artery run into the medial cerebral artery on the same side much more laterally than usual.

These variations naturally do not affect the collateral capacity since they were all complete circles they were just accidentally found anatomic variations.

The data of the smallest diameters of the arterial segments of the circle of Willis:

During our study we determined the average diameters of the different segments and we compared the data of the two sides in case or arteries that are in pairs. We compared the sides because clinical observations indicate that the ischemic strokes are more frequent on the left side.

Statistically relevant side difference was found only in the posterior communicating artery and the first segment of the anterior cerebral artery but in one case the left in the other the right segment's diameter was smaller. We also wanted to know whether there is a difference in the diameters between the sexes. Many arterial segments' smallest diameter is smaller in women than in men (basilar artery, right medial cerebral artery, right posterior cerebral artery, anterior communicating artery).

b. *Application of a mathematical model in assessing the functional ability of the communicating arteries of the circle of Willis*

Autopsy proved general arteriosclerosis in every patient. Patient number 1 had Parkinson's disease, patient no.6 and 12 had intracranial haemorrhage. All the other patients had ischemic stroke which was diagnosed by CT and confirmed by autopsy. The most frequent cause of death was pulmonary embolism (6 out of 16 patients), cardiac insufficiency in 4 (4/16 patients) and brain herniation in 3 (3/16 patients) according to the pathologist.

The results of the transcranial color coded Doppler examinations:

A non-functional ACoA was detected in three patients. In the other 13 cases, ACoAs were considered to be functional based on the carotid compression tests. Functionality of the PCoA's was lacking on both sides in 5 cases on one side in another five cases. Functional PCoAs on both sides were detected in four patients. In two other cases, a fetal posterior cerebral artery was diagnosed.

Mathematical modeling of the data: The 4.1. table shows the three parameters (diameter, length and pressure difference between the two ends) as the determinants of blood flow through a vessel.

4.1. table. Diameters, lengths (both in millimeters) and calculated pressure difference (in mmHg) between the two ends of the communicating arteries (PD).

	ACoA			Right PCoA			Left PCoA		
	Diam	**Lenght**	**PD**	**Diam**	**Lenght**	**PD**	**Diam**	**Lenght**	**PD**
1.	0.5	1.7	4.7	1.3	21	4.3	1.1	16	4.9
2.	0.3	2.3	0.5	0.4	12	14.9	0.6	10	59.7
3.	0.8	2.1	11.3	0.5	17	8.2	0.5	17	39.6
4.	0.4	4.0	47.8	0.5	20	2.0	0.5	20	48.2
5.	1.2	1.8	2.7	0.7	13	7.9	0,6	10	1.6
6.	0.8	1.2	3.6	2.1	15	1.1	0.7	9.0	6.5
7.	1.3	1.0	0.1	0.6	13	2.4	0.5	15	2.5
8.	2.0	2.5	0.1	2.1	19	2.3	1.3	8.0	0.9
9.	0.4	2.0	3.8	0.6	15	1.8	0.6	12	5.1
10.	1.9	1.2	0.3	0.6	19	6.5	1.4	15	2.5
11.	1.6	1.5	0.8	0.6	15	17.7	0.6	13	4.3
12.	0.5	5.5	1.8	1.3	9.0	4.5	0.6	10	5.8
13.	0.8	1.8	10.3	1.3	9.5	0.6	0.4	10.5	15.3
14.	1.3	3.0	0.6	1.8	8.0	1.1	1.9	17	2.5
15.	0.6	4.5	2.1	1.3	8.0	2.0	1.1	12	3.0
16.	0.7	1.8	2.8	0.6	22	10.4	0.5	21	8.2

Calculated blood flow values (in ml/min) through the whole cerebral network (Total)

and through the communicating arteries are shown on table 4.2.

4.2. table. Calculated blood flow values in the segments of the circle of Willis (ml/min)

Afferent vessels

	Right ICA	Left ICA	Right VA	Left VA	Total
1.	336	365	116	104	921
2.	272	0	124	135	531
3.	349	0	154	100	603
4.	203	0	43	104	350
5.	0	445	71	177	693
6.	209	311	103	33	656
7.	284	249	44	168	745
8.	409	412	51	88	960
9.	300	323	142	142	907
10.	417	0	42	225	684
11.	0	456	115	151	722
12.	343	184	169	16	712
13.	378	0	190	40	608
14.	323	442	5	50	820
15.	290	240	25	29	584
16.	160	228	2	150	540

Willisian part

	ACoA	Right PCoA	Left PCoA
1.	11	38	29
2.	2	2	50
3.	143	2	9
4.	20	0	16
5.	209	13	0
6.	94	93	11
7.	10	1	1
8.	67	151	21
9.	3	1	3
10.	145	3	41
11.	213	10	3
12.	1	93	49
13.	168	11	3
14.	42	88	125
15.	5	43	28
16.	35	5	2

Based on TCCD-compression tests, anterior and posterior communicating arteries were divided into two groups: functional and non-functional ones. Diameters of the functional communicating arteries were also significantly higher than those of the non-functional ones.We found that blood flow values were significantly higher in the functional communicating arteries than in the non-functional ones. As expected, blood pressure difference was significantly higher in non-functional communicating arteries than in the functional group.

c. Cerebral vasoreactivity in patients with different severity of carotid stenosis and occlusion

It is obvious that resting cerebral blood flow velocities as measured in the affected and non-affected side were similar in all groups; no side asymmetry was observed.
MCAVs did not differ between the two sides after administration of acetazolamide in the asymptomatic groups. In case of symptomatic stenosis, a marked side difference of the MCAV could be observed after administration of acetazolamide, while in symptomatic occlusions this difference was pronounced only 15 minutes after administering the vasodilatory stimulus.
The percent increase of the MCAV in the asymptomatic groups was similar on both sides at any time after injecting acetazolamide. The same was true for the maximal percent increase of the blood flow velocities (cerebrovascular reserve capacity, CRC). In symptomatic patients, a decreased vasodilatory ability of the cerebral arterioles was detected ipsilateral to the stenotic-occlusive lesion.

Asymmetry index of cerebrovascular reserve capacity:

As mentioned in the methods section, the asymmetry index was calculated by dividing CRC of the affected side by that of the non-affected side.
Thus, an asymmetry index less than 1 indicates a lower maximal vasodilatory capacity of the middle cerebral artery territory ipsilateral to the stenotic-occlusive lesion, whereas an asymmetry index of higher than 1 refers to a more pronounced percent increase of the MCAV contralaterally. Although there is a tendency for all stenotic-occlusive lesions but asymptomatic stenosis to have near 0.5 values, indicating lesser maximal vasodilatory

capacity ipsilateral to the lesion, very obviously, these indices show high standard deviation values. Thus, there should be a wide variation of patients ranging from low asymmetry index to greater than one. This observation is also supported by the minimal and maximal values of this index (asymptomatic stenosis: 0.4–2.92, symptomatic stenosis 0.21–1.29, asymptomatic occlusion _0.97–1.43, symptomatic occlusion _0.17–3.06), indicating that there is a wide individual variation of cerebrovascular reserve capacity. If one takes median values into account, asymptomatic stenosis patients have an index of 1.17, which is near to the expected 1, while all the other patients suffering from stenoses or occlusions have lower medians for asymmetry indices (symptomatic stenosis 0.43, symptomatic occlusions 0.51 and asymptomatic occlusions 0.67), indicating that there is a lower cerebral vasoreactivity ipsilateral to the stenotic-occlusive lesion than on the non-affected side.

5. Discussion

According to the literature the collateral capacity of the circle of Willis plays an important role in case of carotid artery stenosis and occlusion and this is why we performed the morphological analysis of the non-stroke population. Hoksbergen at al. proved with an earlier study that patients who suffer from ischemic stroke have non-functional anterior communicating artery in 33% of the cases and non-functional posterior communicating arteries in 75% of the cases. Both of these percentages are much higher than in the non-stroke population. If the patient has hemodinamically significant carotid stenosis or occlusion in case of non-functional anterior communicating artery the risk of an ischemic stroke is 7 times and in case of non-functional posterior communicating artery 3 times more than if these arteries were functional ones. Japanese authors published similar results and proved that the internal carotid artery occlusion and the insufficient collateral capacity are independent risk factors of ischemic stroke. It has been proved that the characteristics of the intracranial collateral network determine the hemodynamic circumstances that may lead to the rupture of a carotid plaque. As far as the carotid artery reconstructions are concerned according to the literature the collateral capacity has major importance in two cases: in the cross-clamping phase of the endarterectomies and when the clamping is ceased during the revascularisation. More studies proved that in case of incomplete circle of Willis the danger of cerebral ischemia is much greater in the cross-clamping phase of the operation. In the postoperative phase the

most common hemodynamic consequence is the development of the so called hyperperfusion syndrome. There is proof that in case of insufficient collaterals the possibility of the hyperperfusion syndrome is more likely. We can conclude that it is important to know the collaterals of the circle of Willis to be able to judge the danger of perioperative hemodynamic problems. This is why it is important to study the anatomic variations and to get to know the average diameters of the different segments of the circle of Willis in the non-stroke (average) population. According to the literature the frequency of the complete and incomplete circles have a geographic variation and it is influenced by age and sex as well.

In our material we found the incomplete circles to be 22.7 % which correlates with Hoksbergen et all's data from the Netherlands but is much less than in the Indian publication of 54% of incomplete circles. As for the diameters our results completely correlate with the Indian pathological data and the diameters given by MR angiography published by Krabbe-Hartkamp. From the point of view of our further examinations the diameter of the anterior and posterior communicating arteries was the most important. Similarly to earlier investigators these diameters were above 1 mm in the average population.

In a later phase of our study we compared clinical data obtained from transcranial color-coded duplex sonography combined with carotid compression tests to data calculated using a mathematical model of the circle of Willis applied to autopsy data. We demonstrated that non-functional communicating arteries have smaller diameters and carry lower blood flow than functional ones. In addition, our data indicate however, that beside vessel diameters, other factors, such as the pressure difference between the two ends of the communicating arteries, do influence cerebral blood flow through the communicating arteries. Thus, blood flow in collaterals is dependent on the flow and pressure distribution throughout all the vessels forming the circle of Willis. Transcranial color-coded duplex sonography, a recently developed method, enables a precise differentiation of the investigated vessels forming the circle of Willis as it gives a color-coded image of the circle of Willis. The ability of TCCD to assess collateral function of the circle of Willis has been demonstrated in previous studies. Combination of TCCD and carotid compression allows a more precise description of the collateral function. Using adequate patient exclusion criteria carotid compression can be considered a safe procedure of detecting patent collaterals. The advantage of this method is its simplicity, its relatively high sensitivity. Furthermore, TCCD can be performed bedside and therefore it is easy to use during daily clinical practice. The potential disadvantage of the method is that it cannot be used in patients who do not have an adequate temporal bone window, in those who have obstructive lesions at the site of the compression and in patients

with known hypersensitivity of the carotid sinus(es). Thus, TCCD appears as a reliable tool for assessing the clinical applicability of mathematical models for studying functionality of the circle of Willis. The validity of this simulation approach depends on the accuracy of diameter and length assessment and on relevance of the model. In our study, we measured diameters and lengths of the arteries at autopsy, with a precision of 0.1 mm. However, our arterial diameters seem to be underestimated as compared to previous reports while lengths of the arteries as measured by us, are comparable with those of previous investigators. To explain the differences, we have to note, that we prepared circles of Willis from the base of the brain from cadavers, where intraluminar pressure was not present. This is actually one of the major limitations of our and all other previous pathological studies. On the other hand, pressures from outside of vessel lumina (such as intracranial blood pressure), which have a counteraction on normal vessel, were also not present at the time of vessel diameter measurements. The main difference between previous measurements and ours is, that we measured diameters and lengths on native arteries, which were just prepared from the basis of the brain, while others used fixed arterial segments. One of the previous investigators put the fixed circles before measuring diameter into water and thereafter to ethanol, which might have affected the normal elastic properties and therefore diameter measurements. Second, we took only the smallest diameter out of the three into account, as being presumably the main determinant of collateral flow. Third, the majority of our measurements were performed in patients suffering from cerebrovascular diseases in contrast to previous observers, who measured diameters in general patient cohorts. It is known that patients suffering from ischemic stroke have a higher frequency of "thread-like" vessels of the circle of Willis. In our study we found that the diameters of non-functioning collaterals are 0–6 mm in average which was also true for both the anterior communicating and anterior cerebral arteries. These diameters correspond to the previously published morphological and simulation-based data. Using the mathematical simulation model we were also able to show that pressure difference between the two ends of the communicating arteries may also have an impact on the collateral ability of the vessels. This observation is supported by previously published morphological and simulation data. The main limitation of the current mathematical models of brain circulation is that they simulate the peripheral cerebral vascular resistances by using 6 lumped constants which cannot be modified according to those of individual patients at rest and during autoregulation. Consequently, the blood flow values calculated in a given patient are under- or overestimated, depending on the lumped resistances are higher or lower than those of the patient. Although we used lumped vascular resistance values for our calculations, it has

to be noted, that there is lack of reliable and simple formulae enabling calculation of the non-linear resistance of the vascular segment. Furthermore, calculated cerebral blood flow values are not different using linear and non-linear simulation models when all supplying arteries are fully patent . In fact, in pathological conditions the linear model leads to overestimation of flow and pressure rate values at the level of the circle of Willis.

In spite of all these limitations, we were able to treat individual data with a mathematical model and to calculate blood flow and blood pressure values, which are consistent with data obtained by TCCD compression tests and at autopsy. Roughly, both diameter and blood flow were higher in the functional communicating arteries than in the non-functional ones, but the differences in diameter explained poorly the changes in blood flow. Our study points out that blood flow through collaterals do not only depend on the actual vessel diameter, but also on the pressure drop between the two ends of the communicating arteries. This pressure drop is influenced by the pressure distribution within the circle of Willis, and therefore by the diameters of the other vessels and the peripheral resistances as well.

We can conclude that there is no threshold diameter for non-functioning communicating arteries. There is rather a diameter range in which a communicating artery may be either functional or not, depending on other factors besides vessel diameter. The results of the present study and those of previous morphometric analyses and mathematical simulations suggest that this range should spread from 0.5 mm to 1.0 mm. There are many debates about the existence of strokes from hemodynamic origin. In fact, some patients with an internal carotid artery occlusion have a decreased cerebral blood flow and an impaired cerebrovascular reactivity ipsilateral (sometimes contralateral, as a sign of a steal phenomenon) to the obstructive lesion that is believed to be caused at least in part by insufficient collaterals. Some authors described typical brain infarctions (watershed or border-zone infarcts), which is frequently accompanied by insufficient collateral pathways. It is conceivable that with a further advance of non-invasive morphological diagnostic devices (such as magnetic resonance imaging and Doppler equipment), by applying functional tests (such as carotid compression or vasoreactivity tests) and preoperative circulation modeling, a better delineation of a group of patients at higher risk for hemodynamic stroke may become possible. Furthermore, a more precise patient selection based on hemodynamic criteria may improve the effectivity of some revascularisation procedures.

Bearing this in mind we performed cerebral vasoreactivity tests in patients with different severity of carotid artery stenosis and occlusion.

We investigated cerebral blood flow velocities and cerebrovascular reserve capacity values in patients suffering from asymptomatic and symptomatic internal carotid artery stenosis and occlusion. We found that resting blood flow velocities are comparable in the affected and non-affected hemispheres in all groups, but cerebrovascular reserve capacity

is of lower magnitude on the affected side than the non-affected one in all groups but asymptomatic stenosis. Classification of the patients into subgroups of asymptomatic and symptomatic lesions revealed the most important question at the beginning of the

study. A very detailed questionnaire of the previous medical history (including dysesthesias, temporary or permanent weakness of the extremities, speech disturbances, visual symptoms) alone was considered to be uncertain. In our opinion, in some cases temporary circulatory disturbances may be mild and short so the patient does not remember them anymore. Therefore, beside clinical criteria we also introduced morphological criteria for patient selection and only patients with negative CT scans and negative medical history of stroke were classified to the asymptomatic group.

Another methodological point was the use of transcranial Doppler and acetazolamide as a vasodilatory stimulus. Acetazolamide is a widely accepted stimulus for cerebral hemodynamic studies. The drug acts by inhibiting the enzyme carbonic anhydrase, thereby decreasing extracellular pH and increasing extracellular pCO_2. Both decreased pH and increased pCO_2 are patent vasodilators of cerebral arterioles.

Hemodynamically significant stenoses and occlusions of the carotid arteries may result in a pressure drop ipsilateral to the lesion. Several important mechanisms are known to compensate for the hemodynamic compromise caused by extracranial arterial stenosis or occlusion. These pathways are organized in a stepwise fashion: to the first step belong the collaterals of the circle of Willis (anterior and posterior communicating arteries), to the second step the ophthalmic collaterals, and to the third step the leptomeningeal arteries. Finally, if all those collateral compensation capacities become insufficient, the brain tissue is capable of increasing the oxygen extraction from the circulating blood.

An additional pathomechanism includes vasodilation of the cerebral arterioles: after a certain limit of the pressure drop in the brain tissue is reached, an autoregulatory vasodilation of the cerebral arterioles occurs to ensure better tissue perfusion by decreasing vascular resistance. The theoretical background of the ACZ test in stenotic-occlusive lesions is that the pressure drop distal to the lesion induces autoregulatory vasodilation of arterioles. If an autoregulatory vasodilation is present, less or no further vasodilation of the arterioles is possible. Based on the magnitude of the reaction after administering the stimulus, vasodilatory responses can be

categorized into the following groups: 1) reduced augmentation of the blood flow relative to the non-stenostic side, 2) absent augmentation of blood flow, 3) paradoxical reduction of the blood flow compared to the resting value. It is widely accepted that cerebral vasoreactivity reflects the hemodynamic status of the cerebral circulation and that impaired hemodynamics are associated with a higher incidence of TIA or stroke. Symptomatic carotid artery occlusions bring about embolic (from the carotid stump, through the external carotid artery, by transhemispheric passage) and hemodynamic strokes. The yearly incidence of stroke has been found to be between 0 to 20%. It is also clear that impaired or exhausted cerebrovascular reactivity is related to border zone infarcts and indicates poorer prognosis:

the combined stroke incidence is 12.5% if cerebrovascular reactivity response is impaired and 41.4% if exhausted. Based on the investigations of the NASCET study, the cumulative risk of ipsilateral strokes was 26% in symptomatic carotid artery occlusion. There are data indicating that impaired cerebrovascular reactivity measurements of symptomatic patients have a predictive value in identifying patients who need intraoperative shunt insertion and for whom postoperative improvement of the intracerebral hemodynamics can be expected. The significance of cerebral vasoreactivity measurements among asymptomatic patients is still a debated issue. Nighoghossian and co-workers did not find any difference between

the CVR of asymptomatic and that of symptomatic patients, while others found that in patients with asymptomatic stenoses or occlusions, there is a side asymmetry of the CVR. There are authors who found that impaired vasoreactivity correlates with a 100% improvement rate of the hemodynamic status after cerebral revascularisation, and impaired CVR is accompanied by a higher incidence of stroke. In contrast Fu¨rst and co-workers pointed out that in the majority of the asymptomatic patients CVR remains normal. Furthermore, in their study Yonas and co-workers found that an impaired CVR has a predictive value in symptomatic but not in asymptomatic patients.

Comparing these observations with our data, it is obvious that administering the vasodilatory stimulus induced statistically significant changes only in the symptomatic carotid artery occlusions and stenoses in our sample. The same was true for cerebrovascular reactivity (actual percent increase of MCAV at different time points after ACZ). Thus, the major finding of our results is that in patients who had symptomatic effects from carotid stenosis or occlusion, a component of their problem is because of compromised collateral capacity of the circle of Willis, as suggested by the compromised reserve capacity. A similar observation was made by Derdeyn with PET OEF measurements showing that the patients who had raised OEF were also the ones who were more likely to be symptomatic. In asymptomatic patients

the maximal percent increase of the MCAV (CRC) remained comparable on the two sides after injecting the vasodilatory drug. When calculating the side asymmetry of the CRC, only asymptomatic stenosis patients showed a near normal asymmetry index. Although the differences are statistically significant and indicate that a hemodynamic compromise in the ipsilateral hemisphere can be expected in symptomatic stenoses, occlusions, and also in asymptomatic occlusions, it has to be noted that standard deviations are relatively high in all groups. This results in a large individual variability of the data despite a statistically demonstrable difference. Further analysis of the data supports this observation, as steal phenomenon was detected in one and impaired reactivity in two patients in the asymptomatic occlusion group, while in patients suffering from symptomatic occlusions two steal phenomena and six impaired reserve capacity cases were observed. For stenoses, in the asymptomatic groups one impaired, and in the symptomatic six impaired reserve capacities were found. Thus, in individual cases, a hemodynamic compromise of the cerebral circulation may develop, which results in insufficient collateral capacity, and therefore, may necessitate a cerebral revascularisation procedure. Further prospective, randomized studies using hemodynamic criteria for patient selection are needed to clarify whether the clinical efficiency of carotid endarterectomy and extra-intracranial bypass could be improved. Depending on the results of those studies hemodynamic compromise may become an additional and supporting indication for a reconstructive operation or surgical revascularisation.

6. Summary

We have done a morphological analysis of the circle of Willis on a non-stroke population. From the removed hundred and ten circles of Willis we have found twenty five (22,7%) incomplete circles, where due to the absence of one or more arteries the circle is not completely closed.

We have described the anatomic variations of the complete circle of Willises. These anatomic variations did not affect the collateral capacity since they were all complete circles. We determined the average diameter and length of the arterial segments and in case of arteries that are in pairs we compared the diameters of the two sides. We have applied a special flow circulation model based on mathematical formula to examine the collateral function of the circle of Willis.

Based on the premortem performed transcranial colour coded Doppler (TCCD) examinations we classified the collaterals according to function.

Depending on the results of the TCCD we classified the collaterals into two groups: functioning and non- functioning ones. The diameters of the functioning collaterals were significantly higher than that of the non-functioning ones. The calculated blood flow parameters were significantly different in the arterial segments of the circle of Willis in the functioning than in the non-functioning collaterals. We have performed cerebral reactivity tests in case of patients with different severity of internal carotid stenosis and occlusions. With our examinations we intended to find out whether there is a difference in the vasodilator capacity of the cerebral arteries in case of symptomatic and symptom-free patients with haemodinamically significant carotid stenosis and occlusions. There was no difference between the velocities of the two sides in case of resting and there was no side asymmetry in any group. Likewise there was no difference in the flow velocity values between the sides in case of the symptom-free group after the administration of acetazolamide. In the symptomatic group on the contrary there was a significant difference between the two sides 15 minutes after the administration of acetazolamide. We have managed to enter data obtained by TCCD measurements of patients into a lineal fluid model based on mathematics and calculate blood pressure and blood flow velocities. Our results were consentaneous with the result of the TCCD compression tests and the pathological findings. Both the diameters and the velocity values were higher in the functioning than in the non functioning communicating arteries. It is obvious that the

administration of the vasodilatory stimulus made a statistically significant change only in case of the symptomatic carotid artery stenosis and occlusion. The same applies for the cerebrovascular reactivity. A more careful patient selection based on haemodynamical criteria can improve the affectivity of certain vascular surgical operations.

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List of publications related to the dissertation

1. **Orosz, L.**, Hoksbergen, A.W., Molnár, C., Síró, P., Cassot, F., Marc-Vergnes, J., Fülesdi, B.: Clinical applicability of a mathematical model in assessing the functional ability of the communicating arteries of the circle of Willis.
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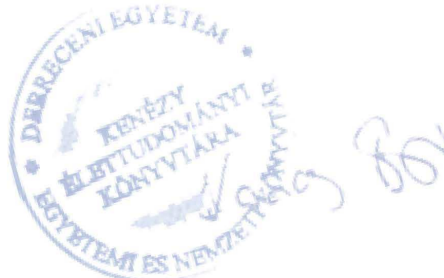
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Total IF of journals (all publications): 3.341

Total IF of journals (publications related to the dissertation): 3.341

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

20 January, 2014