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

Vascular reactivity, vascular wall characteristics and cognitive function in primary hypertension

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Head of the **Examination Committee**:	Miklós Antal MD, PhD, DSc
Members of the Examination Committee:	Zoltán Szabó, MD, PhD
	Attila Valikovics, MD, PhD

The Examination takes place at the Department of Neurology, Faculty of Medicine, University of Debrecen
22nd of December 2014 at 11 a.m.

Head of the **Defense Committee**:	Miklós Antal, MD, PhD, DSc
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The PhD Defense takes place at the Lecture Hall of Bldg. „A", Department of Internal Medicine, Faculty of Medicine, University of Debrecen
22nd of December 2014 at 13 p.m.

1. Introduction and background

1.1. Hypertension

Hypertension is one of the most important modifiable risk factors for cardio- and cerebrovascular diseases resulting in severe target organ damage. The risk for cardiovascular diseases increases continuously as blood pressure (BP) rises from levels that are considered to be within the normal range.

The relationship between blood pressure and the risk for cardiovascular disease is linear and continuous between 115-120/75-80 mmHg and 140/90 mmHg, while over this cutoff value the relationship becomes exponential. The therapeutic target in hypertensive patients is 140/90 mmHg. The benefits of hypertension treatment outweigh its risk and cost beyond the defined target limit of blood pressure.

Compared to international data, the prevalence of cardio- and cerebrovascular diseases in Hungary is high. No matter that cardiovascular disease manifests primarily in the adult age, hypertension often begins in the adolescent, as it was shown in epidemiological studies over the past decades.

The prevalence of adult hypertension is high in Europe and also in the USA. Depending on the age, gender and geographic location, it may vary between 28-36%. The prevalence of high blood pressure is below 10% among patients between 18-35 years of age, is almost 40% in those between 50-59 years of age, while it exceeds 60% over the age of 70. The prevalence of hypertension was 2.53% among adolescents between 15-18 years of age in the Debrecen Hypertension Study. Based on the National Health and Nutrition Examination Survey (NHANES) II, the prevalence of hypertension is higher in men under 50 years of age, is balanced between the two gender in the population aged 55-64, while it is higher in women over the age of 65. These data were confirmed by the Hungarian Hypertension Registry as well.

Hypertension is the most common risk factor for cerebrovascular diseases (acute stroke, hypertensive encephalopathy and vascular dementia). It exerts detrimental effects via damaging the large and small cerebral blood vessels. While major clinical events such as stroke or heart attack usually happen after long periods of uncontrolled hypertension, subtle target-organ damage such as left ventricular hypertrophy, microalbuminuria, or milder cognitive dysfunction takes place early in the course of hypertension.

Clinical trials and observational studies showed that lowering BP to <140/90 mmHg decreases morbidity and mortality, while it improves quality of life by preserving cognitive function. One of the greatest benefits of hypertension control is the reduction of stroke risk, which is the strongest contributor to dementia and cognitive function decline. While serious complications of long lasting high blood pressure are well known and documented, the early alterations caused by hypertension are rarely investigated.

1.2. Hyperlipidemia

Beside hypertension, hypercholesterolemia is another highly prevalent, modifiable risk factor for cerebro- and cardiovascular diseases. Based on a previous observation, if one of the two major risk factors – hypertension or hyperlipidemia – affects a patient, the other risk factor would join earlier and more frequently compared to unaffected patients.

According to Hungarian data, two-third of the adult population has a total cholesterol level above 5.2 mmol/L. Previous studies confirmed that reducing cholesterol level favourably affects total and cardiovascular mortality both in primary and secondary prevention. Other studies pointed out that – in addition to cholesterol – other lipid fractions may also play a role in atherosclerosis development. The Helsinki Heart Study was among the first ones to indicate that elevated level of high-density lipoprotein cholesterol (HDL–C) significantly reduces cardiovascular events. Furthermore, the positive, continuous correlation

between low-density lipoprotein cholesterol (LDL–C) levels and coronary risk is also well known over a broad range of LDL–C levels.

Dyslipidemia, manifested by elevated LDL–C, is central to the development and progression of atherosclerosis. High cholesterol is often a prerequisite for atherosclerotic plaque formation. Based on previous observations, atherosclerosis can already be in an advanced stage without any apparent sign or symptom in hyperlipidemic patients. Consequently, atherosclerosis and dyslipidemia have become primary targets of intervention in strategies for preventing cerebro- and cardiovascular events. Several studies have shown that statins reduce the risk of any cause and coronary mortality, as well as the risk of myocardial infarction, coronary revascularization and ischemic stroke by reducing LDL–C levels.

Of note, large, population-based studies demonstrated that hyperlipidemia, particularly hypercholesterolemia is associated with the risk of subsequent occurrence of mild cognitive impairment, particularly in middle age.

1.3. Cerebro- and cardiovascular reactivity

Although the human brain is only 4.5% of the body weight, it requires 15 to 20% of the whole body content of blood. Constant cerebral blood flow over a broad range of mean arterial pressure (50-150 mmHg) is provided by cerebral autoregulation. By definition, cerebrovascular reactivity means the changes in cerebral blood flow velocity (CBFV) on various vasodilator (breath holding, carbon dioxide inhalation, intravenous administration of acetazolamide) or vasoconstrictor (hyperventilation) stimuli. Changes in the CBFV of basal cerebral arteries induced by different provocation tests can be measured quantitatively by transcranial Doppler (TCD). Persistent hypertension leads to changes in the structure of the cerebral arteries ("remodelling"), and increases arterial stiffness, with subsequent damage of the cerebral autoregulation. The alterations of the brain's microvascular system in asymptomatic hypertensive

patients were extensively studied previously. While some found a reduction in cerebrovascular reactivity of hypertensive patients, others found no difference between hypertensive and control individuals. Recognition of the changes in cerebrovascular reactivity is crucial, as adequate antihypertensive therapy may normalize the impaired cerebral vasoreactivity of patients with early-stage hypertension.

Cardiovascular reactivity is defined as the changes in blood pressure, heart rate and other hemodynamic parameters induced by physical or mental stimuli. There are several possibilities for the evaluation of cardiovascular reactivity under laboratory circumstances: physical activity, changes in body position, simultaneous mental and physical strain, etc. The head-up tilt-table test (HUTT) was first described as a diagnostic test for vasovagal syncope in 1986. Subsequently, it has been widely used in research. Nowadays, it is often used to examine the autonomic nervous system and the cerebral autoregulation. The normal response on HUTT is the relatively stable blood pressure and heart rate between the thirtieth second and the first half an hour following tilting.

1.4. Morphological and functional characteristics of the vascular wall

Elevated blood pressure has been shown to increase the risk for small and large artery lesions, however, several data also suggest that primary and secondary vascular changes (both functional and structural) may precede, or even account for high blood pressure. The two typical types of hypertensive large vessel disease are atherosclerosis and increased arterial stiffness.

1.4.1. Intima-media thickness, as a morphological parameter of the arterial wall

Evaluation of the vascular markers of atherosclerosis allows the recognition of atherosclerosis in its subclinical stage already. The vessel wall can be examined directly, non-invasively with high-resolution B-mode ultrasound, which is one of the best methods for the detection of early stages of

atherosclerotic disease. It is simple, widely available, and depicts the arterial wall structure with a better resolution than any other technique (e.g. magnetic resonance imaging).

Detection of an increased intima-media thickness (IMT) confirms early-stage vascular remodelling. An IMT more than 0.9 mm is a marker of high cerebro- and cardiovascular risk in many guidelines. The process of intima-media thickening is accelerated and enhanced in the presence of atherosclerotic risk factors, particularly hypertension.

Beside dyslipidemia, IMT – as a marker of structural vessel wall property and indicator of hypertensive target organ damage – is another well-described surrogate marker for cerebro- and cardiovascular diseases. Increased IMT has been associated not only with higher prevalence and incidence of coronary artery disease, but also with those of stroke.

Beyond its association with a variety of cerebrovascular and cardiovascular diseases, increased IMT correlated with subsequent cognitive decline in healthy subjects and patients suffering from vascular disease or stroke.

1.4.2. Stiffness parameters, as functional determinants of the arterial wall

Arterial stiffness, the common name for parameters characterizing arterial wall stiffening, is considered to be an independent predictor of cardiovascular- and total morbidity and mortality. In the past decade, the examination of arterial stiffness has been in the focus of clinical attention, which is explained by the widespread use of measuring techniques in the everyday practice, as well as by the growing knowledge regarding the independent prognostic value of some of the parameters of vascular distensibility. From several methods, the determination of augmentation index (AIx) and pulse wave velocity (PWV) became widely utilized to evaluate arterial function. Based on numerous publications in the topic, it became evident that the increase of arterial stiffness and its non-invasively measured parameters (e.g. AIx and PWV) precede the

occurrence of atherosclerosis. Moreover, these parameters were shown to be independent markers of cardiovascular diseases.

AIx is the relative ratio of the difference between the second, reflected (P2) and the first, direct systolic (P1) peaks of the arterial pressure wave and pulse pressure (PP): *AIx = [(P2 - P1) / PP] * 100 (%).*

In addition to the flexibility of the arteries, AIx is determined mainly by the actual peripheral vascular resistance provided by resistance vessels (small arteries, arterioles). The lower the total peripheral resistance, the lower the AIx and vice versa.

PWV is the velocity of the arterial pressure wave between two arterial sites. It is expressed in m/s. The stiffer the vessel, the faster the spreading of the arterial pressure wave, which leads to a higher PWV value. The value of PWV depends on age: in healthy, young individuals its value is between 5-6 m/s, while in the elderly or in conditions significantly reducing arterial stiffness (e.g. renal failure), it may be as high as 12-17 m/s.

PWV is a strong and independent predictor for cardiovascular morbidity in hypertension and diabetes mellitus, and for mortality in hypertension, in patients with end-stage renal disease and in the general population as well. Therefore, evaluation of PWV is recommended for risk assessment in international guidelines for the treatment of hypertension (European Society of Hypertension – ESH, European Society of Cardiology – ESC).

Although the relationship between aortic stiffness and vascular events is continuous, in middle-aged hypertensive patients a threshold of 12 m/s was suggested by the 2007 ESH/ESC guidelines, as an estimate of significant alterations of aortic function. Using the direct carotid-to-femoral distance and taking into account the 20% shorter true anatomical distance travelled by the pressure wave, a recent expert consensus statement adjusted this threshold value to 10 m/s.

Cardiovascular risk assessment can be further refined through age-specific definition of IMT and PWV, and through evaluation of the difference between vascular and chronological age, which may lead to an earlier lifestyle change and medical therapy, if necessary.

1.5. Cognitive function, anxiety and depression

It is well known that hypertension, which is the most common, modifiable risk factor for stroke, is associated with a higher incidence of cognitive decline. The detrimental effect of high blood pressure on neuropsychological performance is well known in the elderly. Based on previous observations, younger hypertensive individuals – as well as older patients – are also susceptible to cognitive decline associated with high blood pressure. The relationship between hypertension and cognitive impairment can be explained by a decreased cerebral blood flow velocity and brain metabolism (impaired glucose utilization) as well as by a disturbed neurochemical transmission. An impaired blood-brain barrier on the basis of damaged vascular endothelium enables access for harmful substances to enter the brain. In addition to hypertension induced atherosclerotic lesions of large vessels, small vessels may also be affected, resulting in silent brain infarctions and white matter lesions (leukoaraiosis). Beyond the structural alterations caused by high blood pressure, hypertension itself is likely to be an independent risk factor for cognitive decline.

Similarly, the association between high BP and anxiety is supported by a large number of case-control studies, which compared either psychological symptoms in hypertensive and control subjects, or BP in patients with a variety of psychiatric disorders and controls. Recently, it was proposed that anxiety could play an important role in hypertension development through altered autonomic control of the heart. While in the NHANES I Epidemiologic Follow-up Study anxiety and depression were predictive of later incidence of hypertension, in another study performed by Paterniti et al. anxiety was

independently-, while depression was not associated with an increased risk for high blood pressure.

Although these findings do not permit the establishment of a causal relationship between anxiety and hypertension, it was suggested that behavioural patterns of anxious patients, such as lifestyle, diets, drinking, smoking or other habits may play a role in high blood pressure development. It was also shown that psychosocial stressors can increase blood pressure and cause anxiety. Anxiety can also cause hypertension through an increased sympathetic activity. Numerous papers in the literature, investigating the link between anxiety and the autonomic nervous system, described the relationship of psychological attributes and the vascular system. Most often psychological factors such as anger and anxiety were associated with hypertension.

2. Aims

Based on the aforementioned findings, the main aims of our research were:

1. To investigate various cardio- and cerebrovascular parameters and their changes during head-up tilt table test in newly diagnosed hypertensive patients, and to subsequently study the cognitive function of these individuals.
2. To evaluate changes in the morphological and functional characteristics of vascular wall in newly diagnosed hypertensive patients in addition to neuropsychological function assessment. We also examined whether the changes are more pronounced when hypertension is combined with hyperlipidemia.

3. Methods

3.1. Subjects

Eighty-one newly diagnosed hypertensive (HT) patients were recruited in our study. Mean age of the patients was 43.5±10.2 [mean±standard deviation (SD)], the male/female ratio was 1.07. None of the enrolled patients had diabetes or other chronic diseases. At baseline examination, two-third of the patients were yet untreated. In more than half of the patients already on treatment, the therapy was started within a week, while none of the remaining patients were treated for more than 2 months. The control group consisted of 94 healthy individuals with a mean age of 44±9.4 years (mean±SD) and a male/female ratio of 1.13. In the second part of our study, to guard against the confounding effects of possible long-standing, asymptomatic blood pressure elevation, patients, in whom explicit target-organ damage could be identified by urine analysis (micro- or macroalbuminuria), echocardiography (left ventricular hypertrophy), cerebral computed tomography (silent brain infarction) or fundoscopic examination (advanced retinopathy), were excluded. From the control group, a few individuals were also excluded, in whom other comorbidities were suspected. Finally, the two groups consisted of 79 hypertensive patients and 87 control individuals. In the second part of our investigations, not only the effect of hypertension, but also that of hyperlipidemia was evaluated. Hence, we included only those subjects in the statistical analysis for the effect of LDL–C, whose serum LDL–C level had been determined (hypertensives: n=72, mean age±SD: 43.60±10.17 years, male/female ratio: 0.95; controls: n=85, mean age±SD: 43.56±8.95 year, male/female ratio: 1.13).

Based on the serum LDL–C level [higher or lower than 3.4 mmol/L (the upper normal limit of LDL–C level according to our laboratory reference values)], the control and hypertensive groups were further divided, resulting in four subgroups: 1) healthy controls, free of hypertension or hyperlipidemia

(CON, *n*=44, mean age±SD: 42.5±9 years, male/female ratio: 1); 2) normotensive subjects with elevated LDL–C levels (LDL, *n*=41, mean age±SD: 44.71±8.86 years, male/female ratio: 1.28); 3) hypertensive patients with normal LDL–C levels (HT, *n*=49, mean age±SD: 41.67±10.29 years, male/female ratio: 0.75); 4) hypertensive patients with elevated LDL–C levels (HT+LDL, *n*=23, mean age±SD: 47.70±8.79 years, male/female ratio: 1.56).

The study was approved by the local Ethical Committee of the University of Debrecen. Informed consent was obtained from all patients and controls.

3.2. Examinations

The following examinations were performed in all groups: laboratory analysis, general physical and neurological examination, head-up tilt table test, neuropsychological evaluation, IMT measurement and assessment of arterial stiffness parameters.

3.2.1. Laboratory analysis

Fasting blood samples were taken for complete blood count, serum glucose, $HbA_{1C,}$ lipids, kidney and liver function and hemostasis. Urine samples were collected in order to exclude micro- or macroalbuminuria.

3.2.2. General physical and neurological examination

General physical and neurological examinations were carried out on all subjects enrolled in the study. With the purpose of data collection blood pressure was measured with an oscillometric device.

3.2.3. Intima-media thickness measurement

High-resolution B-mode carotid ultrasonography was performed using a 7.5 MHz SonoSite MicroMaxx ultrasound machine (SonoSite Inc., Bothell, WA, USA). Measurements were performed in plaque free regions on the far wall of both common carotid arteries (CCAs) approximately 10 mm proximal to the

carotid bulb. On examination R waved triggered longitudinal B-mode images were recorded, saved and stored for later offline analysis. IMT was defined as the distance between the luminal endothelial interface and the junction between the media and adventitia. Six measurements per vessel were performed on both sides, and IMT data of the two CCAs were averaged.

3.2.4. Evaluation of arterial stiffness parameters

Measurements were performed with a validated, computerized portable device (TensioClinic Arteriograph, TL1, TensioMed Ltd., Hungary). During the test the cuff was placed on the dominant upper arm. The principle of the measurement is described in the followings: the device can detect pressure waves in the arterial system by oscillometric technique. The first, direct wave generated by the left ventricular ejection is called P1, while the second wave reflected from the lower body is named P2. The amplitude of the reflected wave depends mainly on the peripheral resistance. AIx can be calculated from the ratio of the difference between wave amplitudes (P2–P1) and pulse pressure (PP). PWV can be determined from the ratio of the distance between the jugulum and symphysis and the reflection time detected by the Arteriograph. The velocity of the generated pressure waves depends on the elasticity of the aorta.

3.2.5. Head-up tilt table test (HUTT)

During HUTT, continuous, simultaneous and non-invasive monitoring of the patients' hemodynamic parameters was performed with the help of a medical device, named Task Force Monitor (CNSystems, Graz, Austria), which applies impedance cardiography, electrocardiography and oscillometric and continuous blood pressure measurements. Bilateral, continuous recordings of the cerebral blood flow velocity (CBFV) of the middle cerebral arteries (MCA) were also obtained with Transcranial Doppler ultrasound, which was attached to the main device. The test lasted for 25 minutes. After 10 minutes of supine (pre-tilt)

position, the patients were passively moved to upright (tilt) position for 10 minutes, as the table was tilted with an angle of 70°. Then, the table and the subject was brought back to horizontal position again (post-tilt), and measurements were continued for another 5 minutes in this setting. During the examination, CBFV (cm/s) was recorded at the right and left MCAs. Blood pressure (mmHg) was measured by two methods. A cuff placed on the right upper arm measured the blood pressure every two minutes with an oscillometric technique. At the same time a continuous ("beat-to-beat") blood pressure monitoring system was also placed on the left forearm, which recorded the blood pressure for each heart cycle. Heart rate was recorded continuously with three-lead electrocardiography. Impedance cardiography allowed monitoring of the following cardiovascular parameters: total peripheral resistance (TPR, dyn*sec /cm^5), stroke volume (SV, ml), cardiac output (CO, l/min) and their indices calculated to body surface area (TPR index – TPRI, stroke index – SI, cardiac index – CI).

During statistical analysis, average values recorded in the different periods of the study (pre-tilt, tilt, post-tilt) were used for all parameters. Cerebrovascular reactivity was defined as changes in CBFV, while cardiovascular reactivity was defined as changes in BP, heart rate (HR) and other hemodynamic parameters in response to tilting.

3.2.6. Neuropsychological performance

All participants completed a one-hour (±10 minutes) neuropsychological test series assessing reaction time, memory, attention, executive function, psychomotor speed, visual-spatial ability, anxiety and depression.

Beside choice- and selective reaction times, missed, wrong and late responses were also recorded. When evaluating verbal learning and memory, the Hungarian version of Rey Auditory Verbal Learning Test (RAVLT) was used. Concentration, selective attention and fluctuation in attention were evaluated

with the Pieron Test. The ability to maintain attention and cognitive processing speed was assessed by Trail Making Test, part A. Digit Span Test evaluated attention and auditory short-term memory. The Block Design Test was used to measure visuospatial and motor skills. Digit Symbol Test evaluated general psychomotor speed and visuomotor coordination. The State-Trait Anxiety Inventory (STAI) included separate questionnaires for state and trait anxiety. State anxiety was defined as a transitory emotional response involving unpleasant feeling of tension and apprehensive thoughts, while the personality trait of anxiety referred to individual differences in the likelihood that a person would have experienced anxiety in a stressful situation. Depression was evaluated by Beck Depression Inventory.

3.2.7. Statistical analysis

When analysing the laboratory variables and HUTT parameters in the first part of our study, unadjusted comparisons were made using t-test and Wilcoxon rank-sum test. Groups were also compared in terms of outcome variables adjusted for sex, age, smoking status (yes/no) and triglyceride level. During analysis of HUTT variables between-phase changes of outcomes within the groups were expressed as percentages. Between-group differences of changes were evaluated using analysis of variance with interaction between stage and group. If earlier-phase outcome levels were found significantly different between groups, the analysis was supplemented by analysis of covariance for the later-phase outcome as well. During evaluation of the neuropsychological performance the previously mentioned statistical methods were used, with the addition of higher education (yes/no) to the adjustment factors. The sum of standardized scores (SSTS) was also modelled against pre-tilt and tilt phase mean BP values [systolic, diastolic BP, mean arterial pressure (MAP)], and against Spielberger state and trait anxiety scores using simple linear regression. In the second part of our study vascular and neuropsychological outcomes were

described using standard statistics, and compared between groups with no adjustment using parametric or nonparametric tests as appropriate for distributional characteristics. Associations between factors and outcomes were assessed using multiple linear regression adjusted for age, sex, smoking status (all models), level of education (neuropsychological outcomes only), MAP (unless BP was an explanatory or outcome variable), and serum LDL–C level (unless lipid level was an explanatory or outcome variable). Effects of categorical factors were expressed as expected values of between-group differences with 95% confidence intervals and P values. Effects of continuous variables were calculated for a single unit increase and expressed similarly.

4. Results I.

4.1. Clinical data of hypertensive patients and control individuals

No differences were found in age, gender, smoking status and the majority of laboratory parameters between the two groups. HT patients had significantly higher body mass indices (P=0.0072) and significantly lower serum carbamide levels (P=0.0006) compared to the control group. In the HT group significantly less patients had higher education compared to the control group (P=0.001).

4.2. Results of head-up tilt table test

In the HT group systolic, diastolic, mean blood pressure and total peripheral resistance index were significantly higher in both supine position and during passive orthostasis. While heart rate of hypertensive patients was significantly higher compared to controls, stroke index was significantly lower during both periods of the test. No differences were detectable in mean (right and left) CBFV of the two groups either in supine or tilted position.

Tilting induced change of parameters did not differ significantly between the two groups. With the exception of cardiac index and CBFV, earlier-phase outcome levels of other parameters were found significantly different between

groups. Thus, analysis of covariance for the later-phase outcome was reassessed after adjustment for earlier-phase outcome readings as-well, which resulted in significant difference in the late phase systolic (P=0.0085), diastolic BP (P=0.0032) and MAP (P=0.0006) and TPRI values (P=0.0189).

4.3. Cognitive performance, anxiety and depression

4.3.1. Results of neuropsychological tests

Hypertensive patients performed significantly worse than controls in short term memory testing (Digit Span Test; P<0.0001). In RAVLT, interaction between hypertension and level of education was present, which led to a difference in test results between the hypertensive patients and the control subjects, with a magnitude depending on the education subgroup. Therefore further subgroup analysis (with or without higher education) was carried out, which revealed a significant difference between the groups without higher education (P=0.0012), however no significant difference could be detected in the group of patients with higher education. Hypertensive patients achieved lower scores than controls in choice reaction time, attention tasks (First Recognition, Pieron Test) and general processing speed (Digit Symbol Test). Although the differences were found to be statistically insignificant in the single tests, the SSTS was significantly lower in the HT group compared to controls both in those with and without higher education (P<0.0001, for both groups).

4.3.2. Results of the inventories assessing anxiety and depression

While in HT patients both state and trait anxiety scores were significantly higher compared to controls (P=0.0432 and P=0.0006, respectively), there were no differences between the groups regarding depression score.

4.3.3. Correlation of neuropsychological tests with blood pressure and anxiety

Generally, the higher the BP values and state and trait anxiety scores, the lower the SSTS during simple linear regression. SSTS correlated negatively with systolic, diastolic BP and MAP values ($P<0.0001$ for all blood pressure values, both in supine position and after tilt) and with state and trait anxiety levels ($P=0.0148$ and $P<0.0001$, for state and trait anxiety, respectively).

5. Results II.

5.1. Clinical data of hypertensive patients and control individuals

There was no difference between the groups regarding gender, smoking habit, fasting blood glucose and creatinine levels. However there was a significant difference between the groups in body mass index, BP values (systolic, diastolic BP, MAP), lipids, and proportion of individuals with higher education.

5.2. Result of intima-media thickness evaluation

Despite IMT being in the normal range in all four groups, during unadjusted comparison IMT was significantly higher in the HT group compared to CON ($P=0.0005$) or LDL groups ($P=0.0142$). A further increase of borderline significance in the IMT value was found when hypertension was associated with elevated LDL–C levels. Adjusted comparison (to age, gender, smoking status and MAP) of subjects with hyperlipidemia (LDL, HT+LDL) to subjects with normolipidemia (CON, HT) revealed no significant difference in IMT values. However, when performing subgroup comparison between hypertensive (HT, HT+LDL) and normotensive subjects (CON, LDL) using multiple regression (after adjustment to age, gender, smoking status and serum LDL–C level), the difference in the IMT values remained significant ($P<0.0001$). While evaluating the adjusted effect of LDL–C level, we found no significant increase in the expected IMT value. However, when analyzing the adjusted effect of MAP, we

found a significant increase of 0.0019 mm in the expected IMT value for each mmHg increase in MAP (P=0.0038).

5.3. Results of stiffness parameter assessments

Raw comparisons revealed a significantly increased AIx value in the HT and LDL groups compared to CON (P=0.0284 and P=0.0311). Hyperlipidemia co-existing with hypertension further increased AIx values, thus comparison between the HT+LDL and CON groups resulted in an even greater difference (P=0.0026). When subjects with hyper- *vs.* normolipidemia were compared using multiple regression, no significant difference was found in the AIx values. In contrast, adjusted comparison of hyper- and normotensive participants revealed a significant difference (P=0.0023).

Unadjusted estimations showed a significant difference between PWV values of CON and HT groups (P=0.0029). Likewise, PWV values were further increased in the group of patients with both risk factors, therefore the differences between HT+LDL and CON or LDL groups were also significant (P<0.0001 and P=0.0019, respectively). Adjusted comparison of subjects with hyper- *vs.* normolipidemia resulted in no significant difference. However, when hyper- and normotensive participants were compared, significantly higher values were observed in association with hypertension (P<0.0001). While analyzing the adjusted effect of LDL–C level on arterial stiffness parameters we found no significant effect. However, MAP-adjusted effect on arterial stiffness parameters showed a significant elevation of 0.50% in the expected value of AIx (P=0.0004), and a significant elevation of 0.058 m/s in the expected value of PWV (P<0.0001) for each mmHg increase in MAP.

5.4. Neuropsychological performance

Raw data showed significant differences between the four groups in Choice Reaction Time, First Recognition, Pieron, Trail Making, Digit Span and Digit

Symbol tests. Adjusted comparison of hyper- and normotensive participants revealed a significant difference in Choice Reaction Time (P=0.0405) and Digit Span Test (P=0.0002). During unadjusted comparison, significant difference in the SSTS could be detected between the CON and HT or HT+LDL groups (P=0.0285 or P=0.0413). Although the adjusted between-groups comparison revealed a strong, but non-significant tendency for hypertensive patients to reach lower scores compared to normotensive subjects, the assessment of the adjusted effect of MAP on SSTS resulted in a significant reduction of 0.1169 in the expected score for each mmHg increase in MAP (P<0.0001).

Neither the comparison between normo- *vs.* hyperlipidemic groups, nor the analysis of the adjusted effect of LDL-C resulted in a significant difference between the groups or in a significant effect.

While the Spielberger state anxiety scores revealed no significant differences between the groups, unadjusted comparison of Spielberger trait inventory revealed significantly higher scores in the HT group compared to CON (P=0.0005). Moreover, a further increase in scores was found, when hypertension associated with elevated LDL–C levels (P=0.0009 or P=0.0456 for HT+LDL vs. CON or LDL, respectively). When analyzing the difference between hyper- and normotensive participants with multiple regression, significantly higher Spielberger trait anxiety scores were observed in hypertensive patients (P=0.0014). Although patients with both risk factors reached the highest score during Beck depression inventory, there was no significant difference between the groups either in unadjusted, or in adjusted models. Effect of MAP and serum LDL-C proved to be unremarkable on depression and anxiety.

6. Discussion

6.1. Vascular reactivity and neuropsychological performance in primary hypertension

The novelty of the present study is that we first investigated the association of early stage hypertension with neuropsychological performance, through testing the cardiovascular-, cerebrovascular reactivity, cognitive function and affectivity on the same population. The main observations of this study are as follows: 1) although no significant differences were found in the cerebrovascular parameters of the two groups during HUTT, in certain cardiovascular parameters significant difference was found in the HT group compared to control individuals; 2) sum of standardized test scores measuring neuropsychological performance were significantly lower in the HT group; 3) state and trait anxiety scores were significantly higher among HT patients compared to controls; 4) inverse correlations of the neuropsychological performances were found with BP values, as well as with anxiety.

The use of head-up tilt table test to induce postural changes in BP and HR has been applied not only for evaluating syncope, but also for studying hypertension. Data regarding cardiovascular reactivity in hypertensive patients are contradictory. Naschitz et al. found a pathological stability of BP and HR during HUTT in hypertensive patients, while Matthews et al. claimed increased reactivity in hypertensive patients and in individuals who are at greater risk for developing hypertension. Tikkakoski et al. observed the following tilt-induced changes in cardiovascular parameters in hypertensive patients: increased cardiac output, less increase in heart rate, less decrease in stroke index and more pronounced increase in TPRI compared to controls.

In the present study, the newly diagnosed hypertensive patients had already higher BP, HR and TPRI levels and lower SI in both supine position and passive orthostasis compared to control individuals. Tilt-induced changes resulted in a

less increase in heart rate, less decrease in SI and a more pronounced increase in TPRI in the hypertensive group compared with normotensive individuals. This observation points out the crucial role of an early diagnosis of hypertension, especially, when the prevention of target organ damage is essential during the long-term therapy. In contrast to the results of the cardiovascular measurements, no changes could be observed in the cerebrovascular parameters of the HT patients compared to controls. These results are consistent with previous literature data, which showed no difference between CBFV of hypertensive and normotensive individuals. While evaluating normotensive and treated hypertensive, elderly patients, Lipsitz et al. observed that despite the tilt induced drop in BP, CBFV remains relatively preserved, demonstrating the integrity of cerebral autoregulation. In addition, in middle-aged and older persons, Eames et al. could not find evidence for the detrimental effect of persistent, untreated hypertension on dynamic or static cerebral autoregulation. Although hypertension may cause early cardiovascular alterations, the relatively longer preserved cerebral autoregulation explains the unremarkable difference in CBFV of hypertensive and normotensive individuals.

While comparing the neurobehavioral performance of hypertensive and normotensive people, Blumenthal et al. found that hypertensive patients performed worse on a set of tasks that measured speed of information processing and short-term memory (Digit Symbol, Digit Span tests and reaction time). Similarly, in our study hypertensive patients reached lower scores compared to control individuals in most of the tests, with the difference being significant for Digit Span Test and RAVLT. The unified test score of the neuropsychological tests also showed a decreased cognitive performance compared to controls, which implies the initiation of a cognitive decline already during the early stages of hypertension. Although these mild changes cannot be observed in an individual's everyday life, they can be interpreted as signs of subsequent cognitive decline related to untreated or inadequately treated hypertension. In

2001 Waldstein et al. found a negative correlation between BP level and cognitive battery in hypertensive patients. Similarly, we also found an inverse correlation between these two parameters in our study population. In 2007 Vetere et al. found a higher anxiety level in primary hypertensive patients compared to normotensive individuals. Likewise, anxiety levels were also higher in our hypertensive patient cohort. Moreover, we found inverse correlation between anxiety and overall neuropsychological performance too. The higher state and trait anxiety scores confirm an altered affectivity of these patients with early-stage hypertension.

In conclusion, our results indicate that there is significant difference in cardiovascular-, but no significant difference in cerebrovascular parameters of newly diagnosed hypertensive patients compared to healthy controls. During neuropsychological testing, hypertensive patients performed significantly worse than control individuals. These changes could be the first symptoms of the long-term effects of hypertension, therefore early and appropriate treatment is required for preventing further impairments. The correlations of cognitive function with the affectivity and BP levels reveal inevitable association between hypertension and neuropsychological performance. Although, the sequence of events is not clear yet, it could be attributable to hypertension induced morphological alterations, as well as to dysfunction in cerebral metabolism and/or neurochemical transmission. In order to clarify the place of events in the time course of the developing pathology, further investigations are needed, which may elucidate the underlying mechanisms behind the above observations.

6.2. Combined effect of hypertension and hyperlipidemia on the morphological and functional characteristics of the vasculature and cognitive function

Beside hypertension, we also investigated the effects of hyperlipidemia on vascular characteristics and neuropsychological performance. Our main

observations from the above measurements are the followings: 1) subtle changes of the vasculature can already be detected in early-stage hypertension; 2) a clear trend was observed in cognitive performance: SSTS was highest in healthy individuals, while lowest scores were reached by patients with both risk factors; 3) degree of anxiety tended to be more pronounced in the presence of risk factors; 4) although hyperlipidemia alone did not impair the examined parameters, when it was associated with hypertension, more pronounced differences could be observed in the assessed parameters.

Pall et al. previously demonstrated that IMT values of the CCA was higher in hypertensive adolescents compared to healthy controls, suggestive of ongoing subclinical target-organ damage in these young patients. Accordingly, we also observed significantly higher IMT values in early-stage hypertensive adults in their mid-forties when compared to control individuals. In addition, Amer et al. recently found that hypertension duration was positively correlated with IMT among senescent hypertensive patients. These findings indicate that hypertension leads to higher IMT values of the arterial wall in all ages.

In our study population, the increasing number of risk factors induced more explicit changes. When analyzing LDL–C adjusted effects, we found no significant differences between the groups. This observation suggests that although hyperlipidemia is definitely a contributing factor to the deterioration of the vascular system, per se it may not yet result in detectable changes at this early stage. In contrast with our results, when Vladimirova-Kitova et al. evaluated IMT in asymptomatic, non-treated, severe hypercholesterolemic subjects, they found that these individuals were at high risk of having increased IMT, especially if endothelial dysfunction was also present. The discrepancy between these observations may originate from the difference in lipid levels: while our patients had only moderately high levels of LDL–C, the population studied by Vladimirova-Kitova et al. had severe hyperlipidemia. In another study, Li et al. observed that over a mean of 10.7 years follow-up, patients with

normal BP but with carotid artery atherosclerosis had a threefold higher risk of ischemic stroke compared to those with normal carotid arteries. These observations emphasize the importance of IMT monitoring, as higher values may draw attention to an increasing stroke risk already in this early stage of atherosclerosis.

Regarding arterial stiffness, Nürnberger et al. previously observed that AIx was correlated with age, diastolic BP, HR, height and gender in a population that was free of any atherosclerotic disease. Similarly, in subjects with atherosclerosis all these parameters were correlated with AIx, with the exception of age. Accordingly, in our study we found higher AIx values in hypertensive subjects after adjustment for parameters such as age, gender, serum LDL–C level and smoking status. At the moment, data regarding the relationship between serum lipid levels and arterial stiffness are controversial. Wilkinson et al. found that patients with hypercholesterolemia had stiffer blood vessels than matched controls, despite their similar peripheral BP values. Although their study showed that stiffness was independently correlated with LDL–C, Nürnberger et al. found no significant association between cholesterol levels and AIx. After all, we could not consistently demonstrate a significant effect of serum LDL-C level on AIx in our study.

Safar et al. stated in 2002 that increased aortic PWV is a strong and independent predictor of cardiovascular risk, regardless of whether this mechanical factor plays a causative role or merely serves as a marker of vascular disease already present. It has been shown in a recent study that PWV at any age is related linearly to systolic-, while symmetrically to any BP level, and is proportional to the square of age. Moreover, after correction for squared age and BP, PWV was not significantly influenced by smoking or lipid status, and gender differences were also negligible. Likewise, in our study PWV was significantly higher in hypertensive subjects compared to normotensive ones during multiple regression analysis (after adjustment to age, gender, smoking

status and serum LDL-C level); however, there was no significant difference between PWV values of normo- *vs.* hyperlipidemic subjects (after adjustment to age, gender, smoking status and MAP). Based on these observations it is likely that the hypertension- and aging related vascular stiffness is independent of hyperlipidemia. Previous studies suggest that stiffening of the arterial wall in hypertensive and/or senescent individuals may derive from mechanisms such as fibrosis or calcification induced vascular changes.

Of note, numerous investigations supported that changes of IMT and arterial stiffness parameters in hypertensive patients can be improved by various antihypertensive therapies.

The relationship between cognitive function and hypertension has been examined by several authors. Debette et al. evaluated the association of vascular risk factor exposure in midlife with cognitive decline in participants without dementia from the prospective Framingham Offspring Cohort Study. They found that hypertension in midlife was associated with a worsening executive function. Accordingly, Knecht et al. found that systolic BP explained up to 11% of the variance in cognitive performance in non-demented groups of individuals in midlife age, suggesting that in this population hypertension may account for one tenth of cognitive impairment and thus for an increased risk for dementia. Another study performed by Vicario et al. demonstrated that cognitive impairment of hypertensive patients is present in areas such as attention, memory, as well as executive function. The above findings are in accordance with our results, as our hypertensive patients reached lower scores particularly in tests evaluating memory and attention. In general, we could not demonstrate any significant effect of adjusted LDL–C on cognitive function. Nonetheless, when calculating the SSTS, substantially lower scores could be observed in hypertensive patients, which became even more explicit when both risk factors were present. After all, these early and subtle changes of neuropsychological parameters cannot be noticed during the everyday life of an individual.

However, they indicate a disturbed cognitive performance in this early stage of hypertension.

Several hypotheses have been previously proposed to provide plausible explanation for the pathomechanism of neuropsychological deterioration in the setting of hypertension: structural vascular changes leading to extracellular oedema, disruption of the blood-brain barrier, chronic cortical de-afferentation resulting in vessel obstruction leading to ischemia, insufficient cerebral blood flow, disturbed cerebral metabolism, autoregulation or neurochemistry, enhanced cardiovascular and neuroendocrine reactivity, anxiety etc. Likewise, the pathophysiology of hyperlipidemia associated cognitive decline has also been thoroughly studied (β-amyloid generation, τ-hyperphosphorylation, inflammation in the brain, etc.), but the exact links between cognitive impairment and these two important cerebro- and cardiovascular risk factors are yet to be clarified. Importantly, the early cognitive deficit of young hypertensive individuals can be reversed with an appropriate antihypertensive therapy, as suggested by previous findings.

The association between high BP and anxiety is supported by a large number of case-control studies. When examining anxiety disorder, Vetere et al. found a higher prevalence in hypertensive individuals compared to controls. Our hypertensive patients also reached higher scores on anxiety inventories. In another study performed by Paterniti et al. anxiety was independently, while depression was not associated with an increased risk for high BP. The relationship between hypertension and anxiety is somewhat similar to the "chicken and the egg" dilemma: it is hard to know which came first. After all, it needs further investigations to elucidate whether patients with hypertension are more susceptible to anxiety, or rather subjects with anxiety tend to develop hypertension over time.

Overall, our results provide insight into the early vascular alterations and cognitive disturbance induced by newly diagnosed hypertension and

hyperlipidemia. We demonstrated that subtle changes in the morphological and functional characteristics of the arterial wall and cognitive performance can already be detected in recently diagnosed hypertensive patients. These fine alterations may be the first signs of the devastating complications of long-standing hypertension. Of note, when hyperlipidemia was associated with hypertension, a more pronounced deterioration of the vasculature could be detected, which underscores the importance of prompt recognition as well as appropriate treatment of both risk factors. These results can particularly be exploited during the everyday clinical practice, where borderline changes of BP or LDL–C levels are often neglected. Our study also points out that monitoring of individuals for high BP and serum lipid levels is essential not only in the apparently ill, but in the seemingly healthy, asymptomatic subjects as well. Education of these individuals facilitates an early alert for their already existing risk factors, and thus, can not only contribute to a successful treatment, but may also prevent further impairments.

7. Summary

The serious complications of long-standing, untreated high blood pressure and high cholesterol level are well known, however, the early, clinically silent changes caused by these risk factors are less documented.

During our investigations we evaluated possible alterations in a variety of cardio- and cerebrovascular parameters, cognitive function, and vascular morphology (intima-media thickness) and function (arterial stiffness) in newly diagnosed hypertensive patients. We also sought for potential worsening of the detectable changes in the setting of hypertension combined with hyperlipidemia.

During our investigations we made the following key observations: 1) while there are no differences in cerebrovascular parameters, significant differences can be observed in certain cardiovascular parameters of newly diagnosed hypertensive patients compared to control individuals during head-up tilt-table testing; 2) patients were found to have a greater susceptibility to anxiety, perform worse on neuropsychological testing, while the changes in their cognitive battery correlate both with blood pressure values and level of anxiety; 3) subtle changes in the morphological and functional characteristics of the patient's vascular wall can already be detected at this early stage of hypertension; 4) further impairment in affectivity, cognitive performance and vascular features can be observed when hyperlipidemia is associated with hypertension.

The above observations highlight not only the importance of an early detection and a prompt, adequate management of hypertension and hyperlipidemia, but they also point out the importance of cognitive impairment evaluation, as well as the significance of an early and complex screening.

8. In extenso publications of the author

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List of publications related to the dissertation

1. **Kovács, K.R.**, Bajkó, Z., Szekeres, C.C., Csapó, K., Oláh, L., Magyar, M.T., Molnár, S., Czuriga, D., Kardos, L., Burainé Bojtor, A., Bereczki, D., Soltész, P., Csiba, L.: Elevated LDL-C combined with hypertension worsens subclinical vascular impairment and cognitive function. *J. Am. Soc. Hypertens. 8* (8), 550-560, 2014.
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IF:2.68 (2013)

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3. Fülesdi, B., **Kovács, K.R.**, Bereczki, D., Bágyi, P., Fekete, I., Csiba, L.: Computed Tomography and Transcranial Doppler Findings in Acute and Subacute Phases of Intracerebral Hemorrhagic Stroke.
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