Age-related cerebrovascular changes: formation of cerebral microbleeds and development of neurovascular uncoupling

DOCTORAL THESIS

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INTRODUCTION

Age-related cerebrovascular changes

Globally there are more than 1 billion people who is over 60 years old and 10% of the word population is over 65 years, and it is still expected to rise in the near future. Even during healthy aging several cellular-, micro-, and macro-cerebrovascular changes appear, which have been linked to age – related cognitive decline, dementia or gait abnormalities, placing an enormous burden on the healthcare systems worldwide. Aging-related cerebrovascular dysregulation includes both structural and functional changes. Among others formation of cerebral microbleeds have been linked to age-related cognitive decline. Also, age-related neurovascular uncoupling, i.e. attenuated increase in blood flow to neuronal activation also contributes to age-related dysfunction of the central nervous system.

Cerebral microbleed formation in aging

Autoregulation and structural changes in aging: basis for formation of CMBs

Autoregulation (AR) stands for a mechanism which aims to secure constant CBF when cerebral perfusion pressure (CPP) changes between 50 – 150 mmHg, by adjusting cerebral vessel diameter to changes in mean arterial pressure (MAP) and cerebral perfusion pressure.
In aging vascular stiffness, atherosclerosis, and impairment of the myogenic response of cerebral vessels result in narrowed AR, leading to hypoperfusion of cerebral tissue when MAP decreases, and hydrostatic overload of the cerebral microcirculation when MAP increases. The hydrostatic overload of the distal part of the cerebral circulation most likely contribute to BBB disruption, extravasation of blood-borne substances into the brain parenchyma, inflammation and CMB formation. Both the prevalence and number of CMBs increase with aging due to increased production and activation of MMPs, increased NF-KB activation causing proinflammatory environment and vessel fragility, atherosclerosis and modified collagen/elastin content of the vascular wall. In addition to the direct effect of aging, the prevalence of other known risk factors of CMBs is increasing by age, such as diabetes mellitus, hypertension, metabolic diseases, or traumatic brain injury (TBI).

CMB formation in TBI and Aging

TBI is the most frequent cause of hospitalization and death in the elderly population causing a global healthcare demand. Mild traumatic brain injury (mTBI), the most frequent form of brain trauma defined as Glasgow Coma Scale (GCS) 14-15, specifically affects elder individuals. Elderly is prone to fall and suffer TBI due to altered vascular adaptation to orthostatic position, frequent vision abnormalities, dehydration, impaired balance and decreased defence mechanisms or compensations against falls due to aged neuro-musculoskeletal system.
After the initial, direct brain injury, TBI induces secondary injury of cerebral tissue. These secondary changes are initiated by oxidative stress, mitochondrial dysfunction, inflammation and redox-dependent activation of MMPs leading to BBB destruction, neurovascular impairment and CMB formation. CMBs or cerebral microhaemorrhages are 5-10 mm diameter hemosiderin accumulations around the cerebral microvessels due to injury-related bleeding of vessels. CMBs have been linked to cognitive decline and are associated with balance and gait dysfunction, further increasing the prevalence of TBI.
Aging is an independent risk factor for CMB formation, as well, partly because of the senescence-related oxidative stress, atherosclerosis, MMP activation, vascular collagen modification and increased fragility together with BBB dysfunction.
The pathobiological pathways which have been demonstrated in age-related CMB formation and TBI-induced development of CMBs are partly identical. Furthermore, aging increases the prevalence of hypertension and decreases the presence and effect of vasoprotective factors (such as insulin like growth factor) exaggerating the vascular damage and complications of TBI.

Hypothesis I

We hypothesized that TBI and aging synergize to induce the formation of cerebral microbleeds, therefore TBI will lead to an increased number of CMBs in the elderly.

Neurovascular coupling and uncoupling

Neurovascular coupling (NVC) is a feed forward mechanism that ensures adequate metabolic supply of activated brain areas by increasing local cerebral blood flow. NVC is precisely controlled by complex signalling mechanisms throughout the NVU. Intact neurovascular coupling is essential for higher cortical functions and requires healthy cerebral vasculature and homeostasis.
NVC is initiated upon neuronal activation via glutamate which acts both on the synapsing neurons, interneurons, endothelial cells and astrocytes. Glutamate

activation will trigger an increase in intracellular Ca^{2+} concentration, resulting in neuronal cyclooxygenase (COX-2) and neuronal nitrogen oxide synthase (nNOS) and phospholipase-A_2 (PLA_2) activation inducing vasoactive substance production such as NO, epoxyeicosatrienoic acids (EETs) and prostanoids, leading to vasodilation.
NVC stands for local hyperaemia of the active neurons, but retrograde propagation of vasodilation on the cerebrovascular tree can be observed mainly mediated by the K^+ currents transmitted via gap junctions or myoendothelial cells.
Altered NVC has been demonstrated in various age-related disorders, and even in normal aging.
Recently secreted anti-geronic factors were identified as important contributors to vascular aging and concomitant neurovascular dysfunction. Insulin-like growth factor (IGF-1) is a liver produced anabolic hormone which has a systematic effect in maintaining healthy body mass, however recent preclinical studies proved critical role on cerebral development and important effects on the neuronal structure and central nervous system plasticity. The level of IGF-1 is significantly decreases with aging due to the age-related decline in GH production contributing to age related cerebromicrovascular dysfunction on a multilevel mechanisms including altered glutamate signalling, defective plasticity and reduced elimination of potentially toxic proteins. Importantly, preclinical studies demonstrate that circulating IGF-1 deficiency in transgenic mouse models impairs both endothelium-mediated and astrocyte-dependent NVC responses, mimicking the aging phenotype. Additionally, disruption of IGF1R signalling specifically in endothelial cells or astrocytes significantly impairs NVC responses in mice, similar to the effects of circulating IGF-1 deficiency and aging. The mechanisms by which IGF-1 deficiency disrupts neurovascular coupling in aging is multifaceted. First, IGF-1 deficiency results in decreased expression of glutamate receptors and transporters in astrocytes leading to decreased glutamate sensitivity upon neuronal activation. Second, IGF-1 deficiency promote disbalance of astrocyte-derived mediators which are important for dilation during NVC, leading to increased vasoconstrictor production. Accordingly, the production of dilator prostaglandins and epoxyeicosatrienoic acids is decreased and the constrictor 20-hydroxytrienoic acid is increased in response to glutamate-stimulation on brain slices isolated from IGF-1 deficient mice, due to an IGF-1 dependent dysregulation of the producing enzymes. IGF-1 deficiency also induces increased production of reactive oxygen species in cerebral endothelial cells, leading to decreased endothelium-dependent dilation of pial and cortical arterioles in the brain. These findings provide strong evidence that circulating IGF-1 modulates NVC responses, however the role of age-related IGF-1 deficiency in neurovascular dysfunction in older adults has not been determined.

Hypothesis II

We hypothesized that age-related IGF-1 deficiency is associated with neurovascular uncoupling in healthy elderly.

METHODS

All studies were carried out at University of Pécs, Neurosurgery Department and approved by National Hungarian and Regional Ethical Committees, registered under 6552–4/2020/EUIG, 7270-PTE 2018.

CMB detection in elderly volunteers and patients after mTBI

Enrolled patients

To evaluate the effect of mTBI on CMB formation in young and elderly we retrospectively analysed the medical history and MRI images of 34 patients who suffered mTBI based on Mayo criteria. As control group 43 aged-matched patients' MRI without TBI in the medical history were analysed, as well.

Detection of CMBs, imaging protocol

To evaluate CMBs susceptibility- and T1-weighted magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and fluid-attenuated inversion recovery (FLAIR) MR images were obtained using 3T MR scanners. For image evaluation, the Slicer software was used.

Microbleed Analysis

The number and location of CMBs were obtained according to the clinically validated Microbleed Anatomic Rating Scale (MARS): (1) infratentorial: brainstem or cerebellum; (2) deep: basal ganglia, thalamus, internal or external capsule, corpus callosum, or either the periventricular or deep white matter; (3) lobar: cortex or subcortical white matter.

Methods to assess neurovascular coupling in elderly volunteers

Enrolled patients

The participants between age 18 – 40 and over 60 years without neurological diseases or any conditions which could affect the IGF-1 level were prospectively enrolled on a voluntary basis. Total of 63 participants were enrolled in this study, with 31 young (mean age: 28.4 ± 4.2 years, 11 females, 20 male) and 32 older adults (mean age: 67.9 ± 4.1 years, 18 females, 14 male).

NVC measurements

To assess NVC responses a transcranial Doppler ultrasound system (TCD) was used with 2 MHz transducers. The probes were manually secured bilaterally to the temporal acoustic window to measure the cerebral blood flow velocity (CBFV) in the middle cerebral arteries (MCAs) at the depth of 45-60 mm. Parallel to the CBFV measurement arterial blood pressure was taken by a finger-cuff non-invasive continuous blood pressure monitor system and a capnograph was used to measure end tidal CO_2.
Mean cerebral blood flow velocity on both sides were recorded continuously in the middle of MCAs in cm/s and integrated to further analyse by ICM+® software. During postprocessing cerebrovascular conductance index (CVCi) was calculated on both sides of MCA as the quotient of CBFv and MAP in cm/s/mmHg to eliminate the effect of blood pressure change on the cerebral blood flow variation.
To analyse the NVC response in MCAs trail making test (TMT) was used. TMT is a neuropsychological assessment, which gives information about visual search, scanning, processing and executive functions based on an easily achieved task. After the installation of the measurement system the baseline CBFV was measured in resting state for 60 seconds (s) followed by the TMT test and further 60 s rest. As a positive control, breath hold test was performed, when the participants were asked to hold their breath for at least 45 s or as long as they can. After another resting state and normalized CBFV and MAP, a negative control test was carried out with hyperventilation for 60 s respectively.

Measurement of serum IGF-1 level

IGF-1 levels were determined by enzyme-amplified chemiluminescence immunoassay using an IGF-1 assay kit.

Magnetic resonance imaging (MRI): flow analysis

MRI measurements were performed on a 3T MRI scanner. Blood flow was measured in the M1 segments of both left and right MCAs using a parasagittal two-dimensional single-slice phase contrast sequence with peripheral pulse gating.

Gait analysis

For the gait analysis a non-invasive marker based dynamic foot pressure measurement and gait analysis system was used. Step lengths (cm), stride lengths (cm), step width in cm, speed of walking (km/h) and percent of the stance phase as well as percent of swing phase were assessed.

RESULTS

Development of CMBs in young and aged patients following mTBI

Study population

77 patients were enrolled to this study and the results were analysed in 4 subgroups: young control (“Y”: n = 20, 10 females, 10 males, age: 25±6 years); young trauma (“Y+mTBI”: n = 17, 11 females, 6 males, age: 25±10 years); aged (“A”: n = 23, 16 females, 7 males, age: 68±5 years); aged trauma (“A+mTBI”: n = 17, 9 females, 8 males, age: 72±7 years). Medical history and cerebrovascular risk factors were evaluated, and there was no difference in the assessed parameters between the study groups.

Aging increased CMB development

In line with previous preclinical and clinical research showing aging is an independent risk factor for CMB formation in our study we found significantly higher number of CMBs in aged participants (Figure 1). We also found that, aging significantly increased the prevalence of microbleeds ($p<0.05$), regardless of the number of lesions in the aged group. Interestingly, mild TBI did not increased significantly the number or the prevalence of CMBs found in ageing (Figure 2).

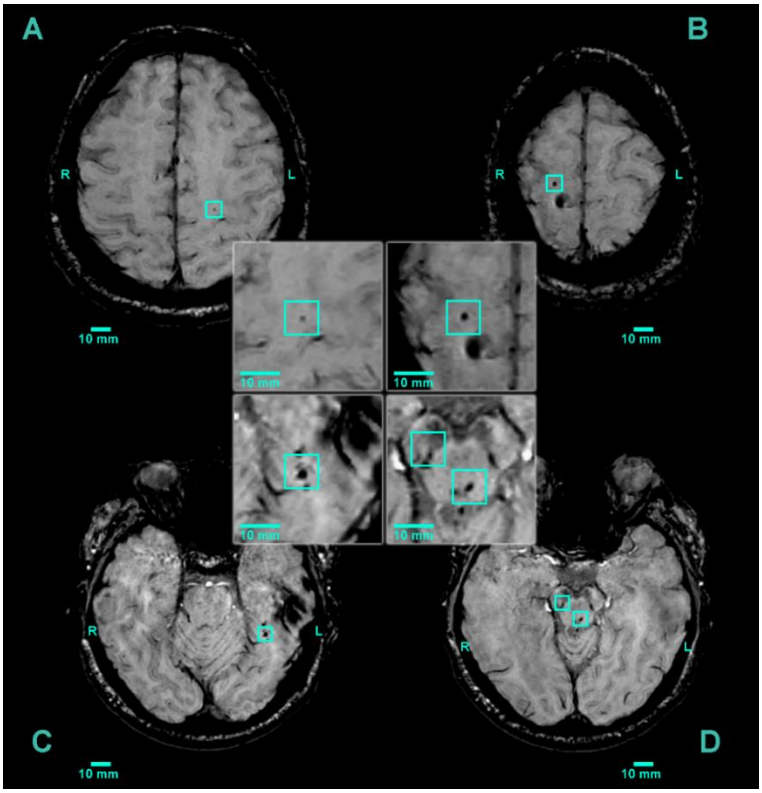

Figure 1 *presents the cerebral microbleeds found on the axial SWI MRI (3 Tesla) in an aged patient, who suffered mild trauma (AT).*
CMBs are highlighted by the blue boxes: ***A****.: left corona radiate* ***B.****: right corona radiate* ***C****.: left parahippocampal gyrus* ***D****.: crus cerebri, medial longitudinal fasciculus*

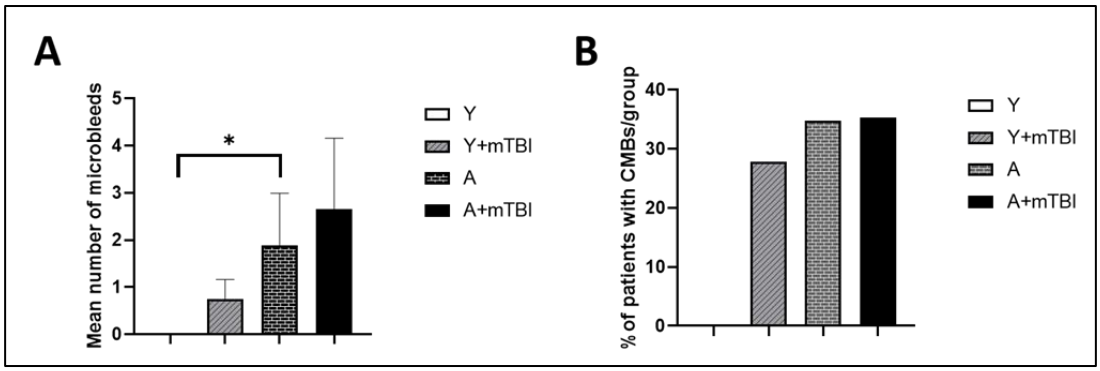

*Figure 2 shows the mean number ±SEM of CMBs found in the evaluated age groups (**A**). The difference between the mean number of CMBs was significant in young vs. aged group. *p<0.05 Panel **B** shows the number of patients with CMB expressed as % of the given group (Y: 0%, Y+mTBI: 27.78%, A: 34.78%, A+mTBI: 35.29%) and A+mTBI was significantly higher than young patients.*

Localisation of CMBs in aged and young patients after mTBI

Further analysis revealed that, most of the CMBs were located in the supratentorial compartment (lobar and basal ganglion) and more specifically aging enhanced the number of parietal and occipital CMBs after an mTBI vs. young mTBI patents. Furthermore an mTBI provoked parietal lobe CMBs in aging compared to young. (Figure 3).

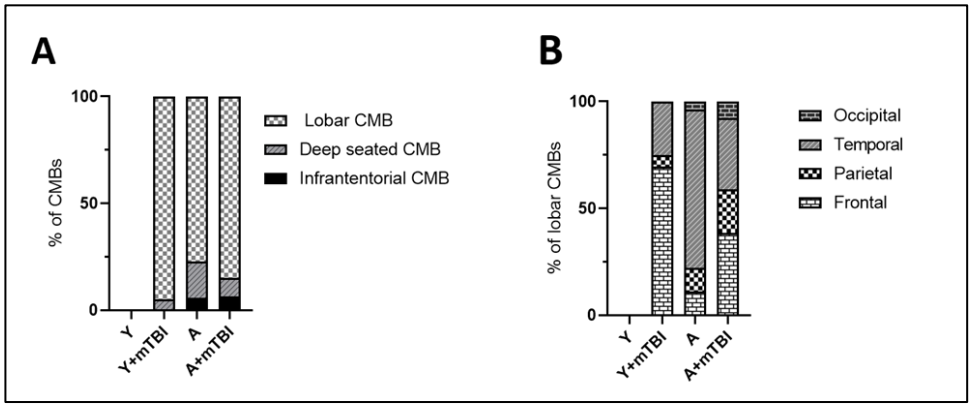

Figure 3 presents the localisation expressed in % of all CMBs in the different study groups based on MARS classification. Lobar CMBs were the most prevalent in all of the study groups regardless of age or TBI. In the aged mTBI group a modest number of CMBs are found in infratentorial localisation compared to aged without trauma, but the difference did not reach significance level. The most frequently seen lobar localisation was further analysed and found that aging increased the number of parietal and occipital CMBs after mild traumatic brain injury (mTBI) (p<0.05 vs. Y+mTBI), and that mTBI leads to the formation of more CMBs in the frontal, parietal and occipital lobes in aging (p<0.05 vs. A).

Age-related neurovascular coupling measurements

MAP and end-tidal CO_2 levels were in the physiologic range during the examinations and there was no significant difference between the two age groups.

Aging decreased basal cerebral blood flow

A subgroup of patients (young n=9, aged n=12) were examined with phase contrast MRI to assess the basal cerebral blood flow in MCAs and significantly decreased mean flow velocities were found in both MCAs. (Figure 4)

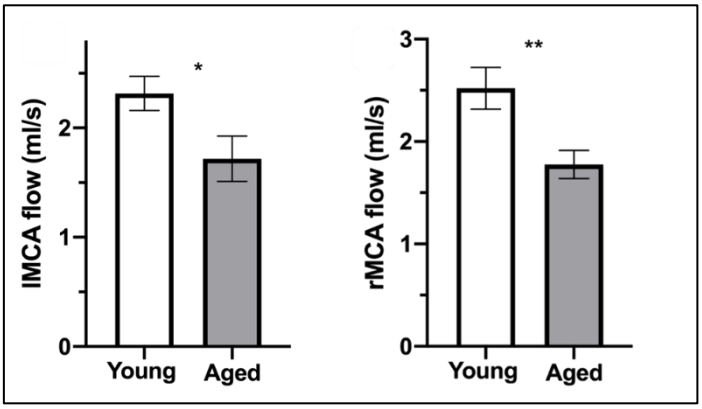

*Figure 4: The mean flow velocity (ml/s) of the left and right MCAs of young (n = 12, left MCA FV: 2.39±0.56 ml/s, right MCA FV: 2.57±0.65 ml/s) and aged volunteers (n = 9, left MCA FV: 1.71±0.65 ml/s, aged right MCA FV: 1.79±0.43 ml/s). Data are mean ± S.E.M.*p < 0.05, **p < 0.003*

Aging altered NVC response

NVC response was expressed in CVCi during the TMT. TMT significantly increased CVCi in left and right MCA in both young (n=31) and aged groups (n=32) and the CVCi % change was significantly higher in young individuals. (Figure 5) Breath hold test was performed as the positive control test and induced significant increase in CVCi in both age groups. (Figure 5)

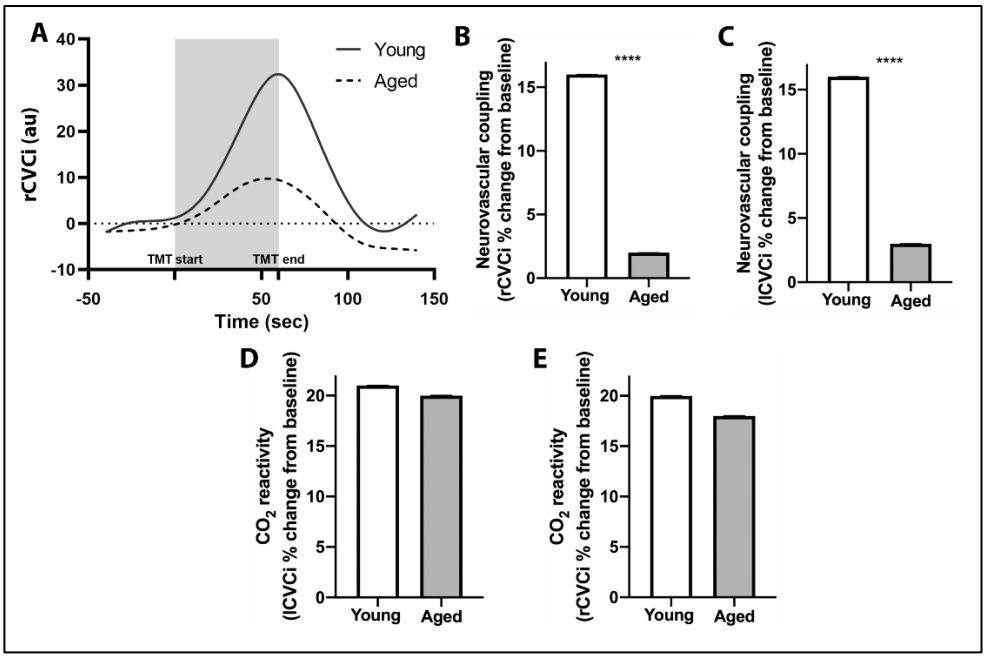

Figure 5 s*hows the impaired neurovascular coupling responses in elder individuals.* ***A:*** *Representative figure of the CVCi changes in the right MCA during TMT in a 25-year-old (young) and a 65-year-old (aged) study participant. The task-evoked maximal NVC response (gray area) in the young and old study participant was 32.4% and 9.7%, respectively, as compared to baseline.* ***B – C:*** *bar graph shows the summation of right (B) and left (C) CVCi change in %±SEM compared to baseline during TMT in young (mean right MCA: 16±0.02, left MCA CVCi: 16±0.02, aged group: right MCA CVCi change: 2±0.01, left MCA CVCi change: 3±0.01). ****p<0.0001* ***D–E*** *CO_2 reactivity as control test in the studied groups, expressed as percentage changes in CVCi in the left (**D**) and right (**E**) middle cerebral arteries during breath-hold test. Data are mean± S.E.M*

IGF-1 level is decreased in the elderly

Serum IGF-1 level was significantly decreased in aged participants compared to young volunteers (young n=31, mean IGF-1 concentration ±S.E.M.: 176.22±8.84 ng/ml vs. aged n=32, mean IGF-1 concentration: 115.70±7.59 ng/ml). (Figure 6)

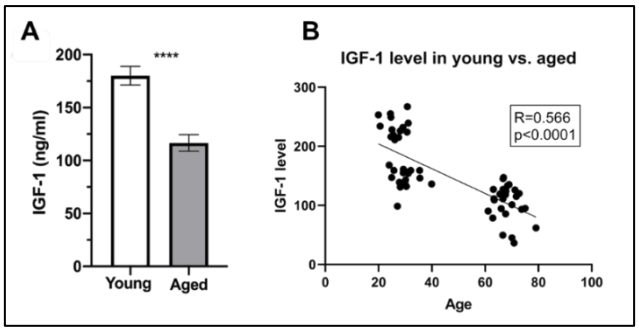

Figure 6 shows serum IGF-1 level in young and aged volunteers. ***A****: IGF-1 is significantly higher in young individuals **** p<0.0001* ***B:*** *Correlation between serum IGF-1 levels (ng/ml) and age (years) in young (n = 31) and aged (n = 32) volunteers*

Decreased IGF-1 level is associated with decreased basal cerebral blood flow

Serum IGF-1 level significantly correlated to basal CBF measured by phase contrast MRI in both of the study groups (R=0.53, p=0.02). (Figure 7)

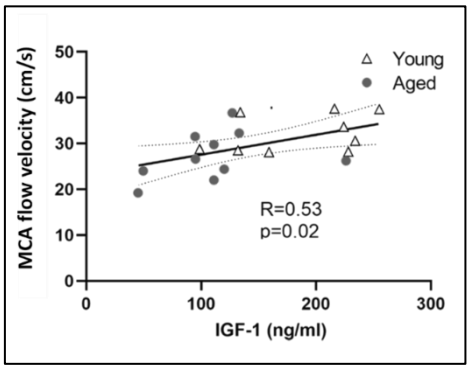

Figure 7 shows the correlation between serum IGF-1 levels (ng/ml) and MCA flow velocity in young and aged volunteers

IGF-1 level correlates with neurovascular uncoupling

Serum IGF-1 levels significantly correlated with changes in CVCi in both MCAs during the TMT compared to baseline CVCi in the studied participants (left MCA CVCi R=0.32, p=0.02, right MCA CVCi: R=0.32, p=0.03), indicating that serum IGF-1 level correlates with neurovascular coupling and its dysfunction in aging (Figure 8).

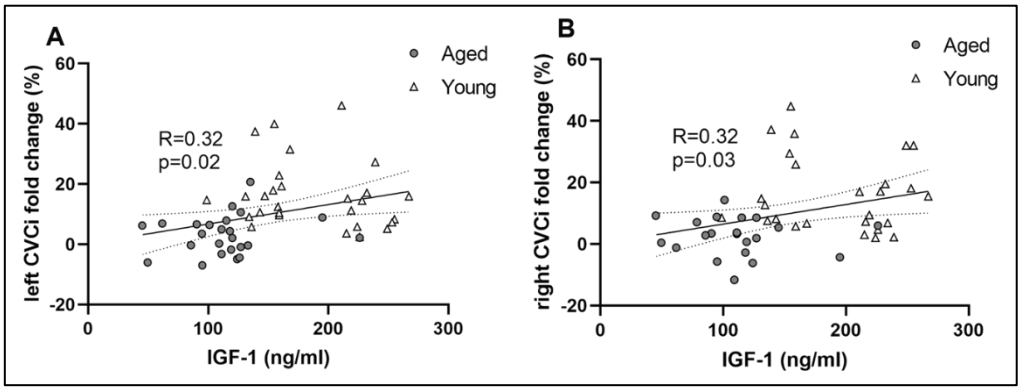

Figure 8 Correlation of CVCi and IGF-1 level in aged and young on the left (A) and right MCA (B)

Gait dysfunction correlates with neurovascular uncoupling and decreased IGF-1 levels in aged individuals

Measurement of dynamic gait analysis showed altered gait functions in elderly compared to the young group. Furthermore, strong correlation was found between mean step length and both % change of CVCi in MCAs during TMT and serum IGF-1 levels in the studied participants, indicating that gait dysfunction correlates with age-related neurovascular uncoupling and age-related decline in serum IGF-1 in humans (Figure 9).

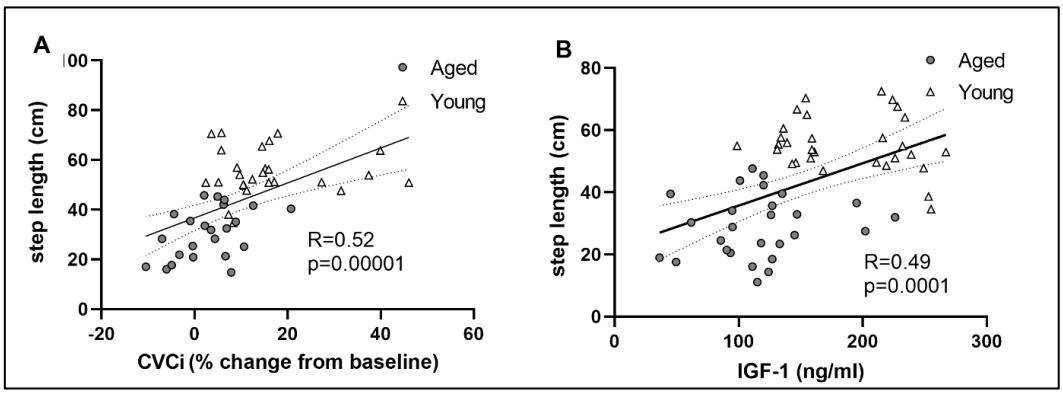

Figure 9 *Age-related gait dysfunction correlates with neurovascular uncoupling and IGF-1 deficiency in humans.* ***A:*** *Correlation between mean step length and % change of CVCi during cognitive test in right MCA of young and aged volunteers (R=0.52, P=0.00001).* ***B:*** *Correlation between mean step length and IGF-1 serum concentration of young and aged volunteers (R=0.49, P= 0.0001).*

DISCUSSION

Formation of CMBs in aging and traumatic brain injury

Confirming previous studies, we found more CMBs in aged vs. young participants, but contrary to our hypothesis we did not detect a significant difference in the number of cerebral microhaemorrhages between aged (A) and aged patients with mild traumatic brain injury (A+mTBI) (Figure 2).
Earlier studies investigated the effects of CMBs on brain function and found that these lesions are not clinically silent, but have been associated with cognitive decline, mental disorders and gait abnormalities, presumably due to the cumulative effect of CMBs, or the anatomical location-related domain specific decline in neurological function, or both. For example, damage of the fronto - subcortical circuits linking prefrontal areas to basal ganglia is associated with impairment in executive function, and disarrangement of pathways from the mentioned areas projecting to thalamus results in memory disturbances. Based on our results, the distribution of CMBs following mTBI are altered in aged patients. (Figure 3) Specifically, we found that the number of occipital and parietal microbleeds were significantly higher in aged following mTBI compared to young participants. This might explain important functional consequences of these CMBs. Accordingly, parietal and occipital lobes have crucial roles in integrating visuospatial and sensory information, cognitive processing, posture and motor control, visual transmission and incorporation, also the visuospatial and cognitive fine tuning of coordination along with rapid movement corrections.
Although it seems logical to posit that (even mild) brain trauma results in enhanced cognitive disturbances in elderly individuals, to our best knowledge, no studies have tested this hypothesis. Thus, future clinical research should investigate the synergistic effect of ageing and TBI-related formation of cerebral microbleeds on cognitive decline.

Age-related neurovascular uncoupling

Here we show that in healthy elderly humans neurovascular coupling responses during cognitive tasks are attenuated compared to young volunteers, confirming observations in laboratory animals. Importantly, we confirm that basal CBF is also decreased by aging, which further decreases the capability of the impaired neurovascular responses in these patients to fulfil metabolic needs of active brain areas by shifting baseline perfusion to lower values.
The mechanisms by which aging impairs neurovascular coupling in humans have not been established, yet. Age related IGF-1 deficiency has been linked to decline of neural function in aging. We recently provided evidence in mice that disrupting IGF-1 signalling in the brain either by adenovirus-associated knock-down of IGF-1 production in the liver or blocking IGF-1 receptors on endothelial cells of cerebral vessels lead to

a significant decrease in neurovascular responses of these animals during neuronal activation, which is associated with both gait and cognitive dysfunction. Based on this here we tested the hypothesis that IGF deficiency is associated with neurovascular uncoupling in elder humans. We show a significant age-related decrease in serum IGF-1 level in the elder group of participants (Figure 6) and demonstrate that IGF-1 levels significantly correlate with neurovascular dysfunction (Figure 8), which strongly suggest that IGF-1 deficiency disrupts neurovascular signalling leading to neurovascular uncoupling in aging humans. Future studies should establish the role IGF-1 related endothelial dysfunction and astrocytic changes (shown in preclinical models) in neurovascular uncoupling in humans and test pharmacological interventions.

As mentioned above, a line of evidence suggest that neurovascular hyperaemia is essential to maintain normal neural function in laboratory animals. Here we show for the first time that gait dysfunction observed in older individuals significantly correlate with neurovascular uncoupling (Figure 9). The causative role of neurovascular dysfunction in the development of neural impairment is strongly suggested by preclinical studies showing that 1) decreased neurovascular coupling is associated with impaired cognitive and gait function in animal models of cerebrovascular risk factors, such as hypertension, diabetes, as well as aging, and 2) direct pharmacological inhibition of neurovascular coupling disrupts normal cognition and gait in mice. This is further supported by our findings that IGF-1 deficiency significantly correlates with gait dysfunction in the studied elder group of participants (Figure 9). Future studies should test whether increasing IGF-1 levels would restore neurovascular coupling, gait and cognitive function in elderly humans.

CONCLUSION, THESES AND PERSPECTIVES

Age-related cerebrovascular changes have been recognized to have great impact on age-related cognitive decline and gait dysfunction, placing an enormous burden on the aging societies worldwide. In the present theses we studied two aspects of age-related cerebrovascular alterations, the formation of cerebral microbleeds in normal aging and after mild traumatic brain injury, and neurovascular dysfunction associated with aging, both of which have been shown to contribute to age-related deterioration of cerebral function.

Thesis 1:

Our results showed that aging leads to enhanced number of cerebral microbleeds compared to young volunteers, and *mild traumatic brain injury and aging do not synergize to increase the number of CMBs*. We found that aging altered the CMB distribution and caused more occipital and temporal lobe lesions in elderly compared to young traumatic patients, which might have an impact on long term consequences and complications of traumatic microbleeds. The major limitations of our studies are the retrospective design and the relatively small sample size. Future prospective studies should verify our findings on a larger number of patients. Also, the possible mechanisms through which aging and TBI may interact to alter cerebrovascular function and formation of CMBs should be studied, with special focus on mitochondrial oxidative stress, activation of redox-sensitive matrix metalloproteinases, modification of the cerebrovascular wall, production of proinflammatory cytokines and disruption of the blood-brain barrier.

Thesis 2:

We showed that *age-related IGF-1 deficiency correlates with aging-induced neurovascular un-coupling and decreased basal cerebral blood flow in humans*, and both of them *correlates with age-related gait dysfunction*. Future studies should establish the possible clinical interventions to restore age-related neurovascular dysfunction to prevent age-related cognitive decline and gait impairment.

Publication list (Impact factor: 59.629)

Publications, the present thesis is directly based on

- Toth L, Czigler A, Horvath P, Kornyei B, Szarka N, Schwarcz A, Ungvari Z, Buki A, Toth P. Traumatic brain injury-induced cerebral microbleeds in the elderly. Geroscience. 2021 Feb;43(1):125-136. doi: 10.1007/s11357-020-00280-3. Epub 2020 Oct 3. PMID: 33011936; PMCID: PMC8050119. (Q1, Impact factor: 7.581)
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Other publications

- Bogár PZ, Tóth L, Rendeki S, Mátyus L, Németh N, Boros M, Nagy B, Nyitrai M, Maróti P. Az egészségügyi szimulációs oktatás jelene és jövője Magyarországon [The present and the future of medical simulation education in Hungary]. Orv Hetil. 2020 Jun;161(26):1078-1087. Hungarian. doi: 10.1556/650.2020.31761. PMID: 32541086.
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