The effect of mild traumatic brain injury on cerebral microbleeds in aging

Luca Toth[1,2], Andras Czigler[1,2], Peter Horvath[1], Nikolett Szarka[2], Balint Kornyei[3], Arnold Toth[3], Attila Schwarcz[1], Zoltan Ungvari[4,5], Andras Buki[1], Peter Toth[1,2,5,6,*]

[1] Department of Neurosurgery, University of Pecs, Medical School, Pecs, Hungary

[2] Institute for Translational Medicine, University of Pecs, Medical School, Pecs, Hungary

[3] Department of Radiology, University of Pecs, Medical School, Pecs, Hungary

[4] Reynolds Oklahoma Center on Aging, Department of Biochemistry, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[5] Department of Public Health, Semmelweis University, Faculty of Medicine, Budapest, Hungary

[6] ELKH-PTE Clinical Neuroscience MR Research Group, Pecs, Hungary

***Correspondence:**
Peter J. Toth M.D., Ph.D
Department of Neurosurgery, University of Pecs
2 Ret Street, Pecs, 7624, Hungary
Phone: +36-72-535-900
Fax: +36-72-535-931
E-mail: toth.peter@pte.hu

Keywords: microhemorrhage, aging, cognitive decline, TBI, microvascular injury

Abstract

Traumatic brain injury (TBI) induces the formation of cerebral microbleeds (CMBs), which are associated with cognitive impairment, psychiatric disorders and gait dysfunction in patients. Elderly people frequently suffer TBI, especially mild brain trauma (mTBI). Interestingly, aging is an independent risk factor for the development of CMBs, as well. However, it is not well established how TBI and aging may interact to promote the development of CMBs. In order to test the hypothesis that mild TBI exacerbates the development of CMBs in the elderly we compared the number and cerebral distribution of CMBs assessed by analysing susceptibility weighted (SWI) magnetic resonance imaging (MRI) in young (25 +/- 10 year-old, n=18) and elder (72 +/- 7 year-old, n=17) patients after mTBI and in aged matched healthy subjects (young: 25 +/- 6 year- old, n=20; aged: 68 +/-5 year-old,

n=23). We found significantly more CMBs in elder patients after mTBI compared to young patients, however, we did not observe a significant difference in the number of cerebral microhemorrhages between aged and aged + mTBI patients. The majority of CMBs were found supratentorially (lobar and basal ganglion). Lobar distribution of supratentorial CMBs showed that aging enhances the formation parietal and occipital CMBs after mTBI. This suggests that aging and mTBI do not synergize in the induction of the development of cerebral microbleeds and that different distribution of mTBI-induced CMBs in aged patients may lead to specific age-related clinical characteristics of mTBI.

1 Introduction

Traumatic brain injury (TBI) has been shown to induce the formation of cerebral microbleeds (CMBs) (1-6). CMBs are hemosiderin deposits of 5 to 10 mm in diameter resulting from bleeding from injured small cerebral arteries, arterioles or capillaries, which are associated with the development of cognitive impairment, psychiatric disorders and gait dysfunction (1, 3, 5-14). Due to orthostatic hypotension, dehydration and impaired balance the elderly population frequently suffers TBI (3, 15, 16). The most common form of TBI affecting elderly people is mild brain trauma (mTBI) (15-17). Similarly to TBI, aging is an independent risk factor for the development of CMBs, as well (3, 5, 6, 10). The number of CMBs increases with age, and they are causally linked to age-related cognitive decline and gait disturbances. Interestingly, mechanisms leading to the formation of CMBs, such as cerebrovascular oxidative stress, activation of matrix metalloproteinases, modification of the content of the cerebrovascular wall, are all induced by both aging and TBI (4, 6, 12, 14, 17-20). However, it is not well established and characterized how TBI and aging interact to promote the development of CMBs, especially after mild brain trauma. In this brief study we tested the hypothesis that mild TBI exacerbates the development of CMBs in the elderly compared to young patients, and aimed to characterize the location and distribution of CMBs in elder patients after mTBI.

2 Materials and Methods

2.1 Study population

The study was approved by the Regional Ethic Committee of the University of Pecs, Medical School, Hungary (7270-PTE 2018). We retrospectively analysed the medical history and 3 Tesla susceptibility weighted (SWI) MRI of 35 patients' (15 males, 20 females), who had suffered mTBI

(GCS 14-15) and were admitted to the Department of Neurosurgery, Medical School, University of Pecs, Hungary between April of 2014 – September of 2019. We also analysed the SWI MRI images of 43 aged matched control patients (17 males and 26 female) without TBI in medical history. For the TBI groups the inclusion criteria were: young: age between 18 – 40 years, aged: above 60 years old at the time of the injury; mild TBI in the history within 6 months to MRI; mild TBI according to Mayo criteria: GCS 14-15, absence or maximum 30 minutes of loss of consciousness, absence of posttraumatic amnesia (PTA) (21). Exclusion criteria: any conditions associated with CMB formation in the medical history as: epilepsy, previous TBI, stroke, transient ischaemic attack, cavernous malformations, cerebral amyloid angiopathy, chronic hypertensive encephalopathy, acute haemorrhagic leukoencephalitis, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Alzheimer disease, cerebral vasculitis, cerebral metastases, haemorrhagic micrometastases, intracranial embolism, intravascular lymphoma, posterior reversible encephalopathy syndrome (PRES), progressive facial hemiatrophy, thrombotic microangiopathies, intracranial infection, COL4A1 brain small-vessel disease (6, 22, 23). For the control group additional exclusion criteria was TBI in the medical records. Both in the TBI and control group, two age groups were defined in a 2x2 study design: young (Y): n=20, 10 females, 10 males, age: 25 +/- 6 years; young + mTBI (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 + mTBI (A+mTBI): n=17, 9 females, 8 males, age: 72 +/- 7 years..

2.2 Imaging protocol

Brain MRI was performed using 3T (Magnetom Trio/Prismafit) Siemens MR scanners. SWI, T1-weighted magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and Fluid-attenuated inversion recovery (FLAIR) images were obtained. T1-weighted high-resolution images were obtained using a three-dimensional (3D) MP-RAGE sequence (TI=900-1100 ms; TR=1900-2530 ms; TE= 2.5-2.4 ms; slice thickness=0.9-1.0 mm; field of view (FOV) = 256 mm*256 mm; matrix size = 256*256, 3D SWI images were acquired as follows: TR=27-49 ms; TE= 20-40 ms; slice thickness=1.2-3.0 mm; FOV= 137-201mm*230-240 mm; matrix size= 125-182* 256-320, with no inter-slice gap. For image evaluation 3D Slicer 4.8.1 (http://www.slicer.org) software was used.

2.3 Microbleed analysis

Three independent neuroradiologists evaluated the images individually, blinded to medical history. In order to precisely identify CMBs, exclusion of SWI lesions that mimic CMBs (intersection

of veins, bottom of sulci, calcium deposits, artefacts caused by air-tissue interfaces or macroscopic bleeding caused by e.g., an intraventricular drain) was carried out (22, 23). The number and location of CMBs were obtained, according to the clinically validated Microbleed Anatomic Rating Scale (MARS) (24). MARS distinguishes the number of definite and possible lesions and precisely localises the CMBs according to anatomic regions as follows: 1) infratentorial: brainstem or cerebellum; 2) deep: basal ganglia, thalamus, internal or external capsule, corpus callosum, either the periventricular or deep white matter, and 3) lobar: cortex or subcortical white matter. In this study we present only the definite lesions (Fig.1).

2.4 Statistical analysis

Kolmogorov-Smirnov test was used to determine whether sample data have the characteristics of a normal distribution. In order to compare the presence of microbleeds, the number of lesions and specific distribution in different sample groups Kruskal-Wallis with post hoc Dunn's Multiple Comparison Tests and Mann-Whitney U tests were used. To evaluate the effect of comorbidities on number of CMBs, Fisher's exact tests were applied. Differences were considered significant at $p<0.05$. Statistical analysis was performed using Origin Pro 2018 software.

3 Results

3.1 The effect of mild traumatic brain injury on the formation of cerebral microbleeds in aging

Characteristics of patients in each group are shown in Table 1. There were no differences in the assessed cerebrovascular risk factors between the groups.

We found that aging exacerbated the formation of CMBs significantly ($p<0.05$) compared to young patients (Fig 2A) confirming the results of previous studies showing that aging is an independent risk factor for the development of CMBs (3, 10, 22). Importantly, the number of CMBs in elder patients was not further increased by mTBI (Fig 2A). mTBI did not enhance the number of CMBs in young patients, either (Fig 2A). We found that aging also exacerbated significantly ($p<0.05$) the incidence of patients with CMBs regardless the number of bleedings (per cent of patients with CMBs in the given group of patients) compared to young patients (Fig 2B), which was not affected by mTBI (Fig 2B).

3.2 Location characteristics of aging and mild traumatic brain injury-induced cerebral microbleeds

We found the majority of CMBs in the supratentorial compartment (lobar and basal ganglion), however a small number of microbleeds appears in infratentorial location in aged patient after mTBI. The difference did not reach statistical significance (Figure 2C). Analyzing the distribution of supratentorial CMBs across cerebral lobes (frontal, temporal, parietal, occipital) we found that aging enhances the number of parietal and occipital CMBs after mTBI ($P<0.05$ vs. Y+mTBI), and that mTBI leads to the formation of more CMBs in the parietal lobes in aging ($P<0.05$ vs. A) (Figure 2D).

4 Discussion

It has been shown previously that both TBI and aging induces the development of CMBs (1, 3, 4, 6, 8, 10). In both cases CMBs are associated with long term cognitive deficit and gait dysfunction and determine the outcome of patients (1, 3, 5-7, 10-14, 23). Previous epidemiological studies proposed that TBI-related development of CMBs is exacerbated in aging (3). However, the effect of mTBI on the development of CMBs in aging, which is the most frequent form of brain trauma, has not been established (9, 15, 16). Here we show (Figure 2) that significantly more microbleeds can be found in the aging human brain than in young healthy individuals, confirming the results of previous studies (7, 8, 10, 14, 22). We also found significantly more CMBs in elder patients after mTBI compared to young mTBI patients, however, we did not observe a significant difference in the number of cerebral microhemorrhages between aged and aged + mTBI patients. This suggests that aging and mTBI do not synergize in the induction of the development of CMBs.

Clinical consequences of CMBs, such as the development of cognitive decline is most likely due to the cumulative effects of the lesions as well as damage in specific anatomical locations (6, 14, 25). For example, damage of 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 (14, 25, 26). Although morphological characteristics based on MRI examination are not helpful to distinguish between CMBs of different etiologies, specific locations suggest the pathophysiological reasons of CMBs formation (6, 9, 22, 23, 25). For example typical brain areas for traumatic CMBs are corona radiata and longitudinal fasciculus (5, 6, 9). Cerebral microbleeds in deep cerebral areas are thought to be due to cerebral angiopathy induced by hypertension, and lobar CMBs are likely due to amyloid angiopathy (6, 9, 23, 25). We found that aging alters the distribution of CMBs after mTBI (Figure 2). Namely, in elder patients following mTBI the number of occipital and parietal bleedings were exacerbated compared to young patients. This may affect the functional consequences of these bleedings. Accordingly, occipital and

parietal lobes are responsible for integrating visual and cognitive information, playing an important role in voluntary coordination, posture and motor control, spatial cognition and rapid corrections of movements (27-30). Specific tests should be part of patient characterization after mTBI to assess the region-specific consequences of CMBs in aging (and young patients, as well), such as the trail making test, the Beck's depression test, Montreal Cognitive Assessment test etc. This possibility should be verified in the future.

Limitations and perspectives

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 large number of control healthy volunteers. We used the Mayo criteria to define mTBI. Since other guidelines suggest slightly different scoring systems, it would be important to compare CMB formation in TBI groups defined by various scoring systems. Aging and mTBI may interact in altering regulatory mechanisms of cerebral blood flow (CBF) in a functional manner. Accordingly, changes in neurovascular coupling, autoregulation of CBF and cerebrovascular reactivity should be assessed and correlated with cognitive and gait function in different age groups after mTBI. Finally, 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 (5, 10-12, 14, 20, 22, 23, 26).

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

LT and PT designed studies and protocols, AC, PH, BK and NS performed literature search and collected patient data, LT, AC, BK, AT performed image analysis, LT, AC and NS generated figures, LT, AC and PT wrote the manuscript, LT, AC, PH, BK, NS, BK, AT, AS, ZU, AB, TP edited and revised the manuscript.

7 Funding

This work was supported by grants from the National Research, Development and Innovation Office to PT (NKFI-FK123798) and AB (K-134555), the Hungarian Academy of Sciences Bolyai Research Scholarship to PT, ÚNKP-20-3-II-PTE-493 New National Excellence Program of the Ministry for Innovation and Technology to PT and LT, EFOP-3.6.2.-16-2017-00008, GINOP-2.3.2-15-2016-

00048, GINOP-2.2.1-15-2017-00067 to PT and AB, Hungarian Brain Research Program 2.0 Grant No. 2017-1.2.1-NKP-2017-00002 to AB, Thematic Excellence Program 2020-4.1.1-TKP2020 National Excellence Sub-program to LT, the Higher Education Institutional Excellence Programme of the Ministry of Human Capacities –to PT and AB, the National Institute of Health R01-AG055395, R01-NS100782, R01-AT006526 to ZU.

8 Reference

1. Huang YL, Kuo YS, Tseng YC, Chen DY, Chiu WT, Chen CJ. Susceptibility-weighted MRI in mild traumatic brain injury. *Neurology* (2015) 84(6):580-5. Epub 2015/01/13. doi: 10.1212/wnl.0000000000001237. PubMed PMID: 25576634.
2. Toth A, Kovacs N, Tamas V, Kornyei B, Nagy M, Horvath A, et al. Microbleeds may expand acutely after traumatic brain injury. *Neuroscience Letters* (2016) 617:207-12. doi: https://doi.org/10.1016/j.neulet.2016.02.028.
3. Irimia A, Van Horn JD, Vespa PM. Cerebral microhemorrhages due to traumatic brain injury and their effects on the aging human brain. *Neurobiology of aging* (2018) 66:158-64. Epub 2018/03/27. doi: 10.1016/j.neurobiolaging.2018.02.026. PubMed PMID: 29579686; PubMed Central PMCID: PMCPMC5924627.
4. Griffin AD, Turtzo LC, Parikh GY, Tolpygo A, Lodato Z, Moses AD, et al. Traumatic microbleeds suggest vascular injury and predict disability in traumatic brain injury. *Brain* (2019) 142(11):3550-64. doi: 10.1093/brain/awz290 %J Brain.
5. Toth L, Czigler A, Horvath P, Kornyei B, Szarka N, Schwarcz A, et al. Traumatic brain injury-induced cerebral microbleeds in the elderly. *GeroScience* (2020). Epub 2020/10/05. doi: 10.1007/s11357-020-00280-3. PubMed PMID: 33011936.
6. Ungvari Z, Tarantini S, Kirkpatrick AC, Csiszar A, Prodan CI. Cerebral microhemorrhages: mechanisms, consequences, and prevention. *American journal of physiology Heart and circulatory physiology* (2017) 312(6):H1128-h43. Epub 2017/03/21. doi: 10.1152/ajpheart.00780.2016. PubMed PMID: 28314762; PubMed Central PMCID: PMCPMC5495931.
7. Akoudad S, Wolters FJ, Viswanathan A, de Bruijn RF, van der Lugt A, Hofman A, et al. Association of Cerebral Microbleeds With Cognitive Decline and Dementia. *JAMA neurology* (2016) 73(8):934-43. Epub 2016/06/09. doi: 10.1001/jamaneurol.2016.1017. PubMed PMID: 27271785; PubMed Central PMCID: PMCPMC5966721.
8. Wang R, Liu K, Ye X, Yan S. Association Between Cerebral Microbleeds and Depression in the General Elderly Population: A Meta-Analysis. *Frontiers in psychiatry* (2018) 9:94. Epub 2018/04/05. doi: 10.3389/fpsyt.2018.00094. PubMed PMID: 29615939; PubMed Central PMCID: PMCPMC5868197.
9. Wang X, Wei XE, Li MH, Li WB, Zhou YJ, Zhang B, et al. Microbleeds on susceptibility-weighted MRI in depressive and non-depressive patients after mild traumatic brain injury. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* (2014) 35(10):1533-9. Epub 2014/04/18. doi: 10.1007/s10072-014-1788-3. PubMed PMID: 24740482.
10. Toth P, Tarantini S, Springo Z, Tucsek Z, Gautam T, Giles CB, et al. Aging exacerbates hypertension-induced cerebral microhemorrhages in mice: role of resveratrol treatment in vasoprotection. *Aging cell* (2015) 14(3):400-8. Epub 2015/02/14. doi: 10.1111/acel.12315. PubMed PMID: 25677910; PubMed Central PMCID: PMCPMC4406669.
11. de Laat KF, van den Berg HA, van Norden AG, Gons RA, Olde Rikkert MG, de Leeuw FE. Microbleeds are independently related to gait disturbances in elderly individuals with cerebral small vessel disease. *Stroke* (2011) 42(2):494-7. Epub 2010/12/18. doi: 10.1161/strokeaha.110.596122. PubMed PMID: 21164137.

12. Ungvari Z, Yabluchanskiy A, Tarantini S, Toth P, Kirkpatrick AC, Csiszar A, et al. Repeated Valsalva maneuvers promote symptomatic manifestations of cerebral microhemorrhages: implications for the pathogenesis of vascular cognitive impairment in older adults. *GeroScience* (2018) 40(5-6):485-96. Epub 2018/10/04. doi: 10.1007/s11357-018-0044-9. PubMed PMID: 30288646.
13. Nyúl-Tóth Á, Tarantini S, Kiss T, Toth P, Galvan V, Tarantini A, et al. Increases in hypertension-induced cerebral microhemorrhages exacerbate gait dysfunction in a mouse model of Alzheimer's disease. *GeroScience* (2020) 42(6):1685-98. doi: 10.1007/s11357-020-00256-3.
14. Werring DJ, Gregoire SM, Cipolotti L. Cerebral microbleeds and vascular cognitive impairment. *Journal of the neurological sciences* (2010) 299(1-2):131-5. Epub 2010/09/21. doi: 10.1016/j.jns.2010.08.034. PubMed PMID: 20850134.
15. Rauen K, Späni CB, Tartaglia MC, Ferretti MT, Reichelt L, Probst P, et al. Quality of life after traumatic brain injury: a cross-sectional analysis uncovers age- and sex-related differences over the adult life span. *GeroScience* (2020). doi: 10.1007/s11357-020-00273-2.
16. Krishnamoorthy V, Distelhorst JT, Vavilala MS, Thompson H. Traumatic Brain Injury in the Elderly: Burden, Risk Factors, and Prevention. *Journal of trauma nursing : the official journal of the Society of Trauma Nurses* (2015) 22(4):204-8; quiz E3-4. Epub 2015/07/15. doi: 10.1097/jtn.0000000000000135. PubMed PMID: 26165873.
17. Rachmany L, Tweedie D, Li Y, Rubovitch V, Holloway HW, Miller J, et al. Exendin-4 induced glucagon-like peptide-1 receptor activation reverses behavioral impairments of mild traumatic brain injury in mice. *AGE* (2013) 35(5):1621-36. doi: 10.1007/s11357-012-9464-0.
18. Go V, Bowley BGE, Pessina MA, Zhang ZG, Chopp M, Finklestein SP, et al. Extracellular vesicles from mesenchymal stem cells reduce microglial-mediated neuroinflammation after cortical injury in aged Rhesus monkeys. *GeroScience* (2020) 42(1):1-17. doi: 10.1007/s11357-019-00115-w.
19. Abdul-Muneer PM, Pfister BJ, Haorah J, Chandra N. Role of Matrix Metalloproteinases in the Pathogenesis of Traumatic Brain Injury. *Molecular neurobiology* (2016) 53(9):6106-23. Epub 2016/11/01. doi: 10.1007/s12035-015-9520-8. PubMed PMID: 26541883.
20. Lewén A, Fujimura M, Sugawara T, Matz P, Copin JC, Chan PH. Oxidative stress-dependent release of mitochondrial cytochrome c after traumatic brain injury. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* (2001) 21(8):914-20. Epub 2001/08/07. doi: 10.1097/00004647-200108000-00003. PubMed PMID: 11487726.
21. Malec JF, Brown AW, Leibson CL, Flaada JT, Mandrekar JN, Diehl NN, et al. The mayo classification system for traumatic brain injury severity. *Journal of neurotrauma* (2007) 24(9):1417-24. Epub 2007/09/26. doi: 10.1089/neu.2006.0245. PubMed PMID: 17892404.
22. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* (2009) 8(2):165-74. Epub 2009/01/24. doi: 10.1016/s1474-4422(09)70013-4. PubMed PMID: 19161908; PubMed Central PMCID: PMCPMC3414436.
23. Yakushiji Y. Cerebral Microbleeds: Detection, Associations and Clinical Implications. *Frontiers of neurology and neuroscience* (2015) 37:78-92. Epub 2015/11/21. doi: 10.1159/000437115. PubMed PMID: 26587900.
24. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jäger HR, et al. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* (2009) 73(21):1759-66. Epub 2009/11/26. doi: 10.1212/WNL.0b013e3181c34a7d. PubMed PMID: 19933977.
25. Ding J, Sigurðsson S, Jónsson PV, Eiriksdottir G, Meirelles O, Kjartansson O, et al. Space and location of cerebral microbleeds, cognitive decline, and dementia in the community. *Neurology* (2017) 88(22):2089-97. Epub 2017/05/05. doi: 10.1212/wnl.0000000000003983. PubMed PMID: 28468844; PubMed Central PMCID: PMCPMC5447401.

26. Levit A, Hachinski V, Whitehead SN. Neurovascular unit dysregulation, white matter disease, and executive dysfunction: the shared triad of vascular cognitive impairment and Alzheimer disease. *GeroScience* (2020) 42(2):445-65. doi: 10.1007/s11357-020-00164-6.
27. Niogi SN, Mukherjee P, Ghajar J, Johnson C, Kolster RA, Sarkar R, et al. Extent of microstructural white matter injury in postconcussive syndrome correlates with impaired cognitive reaction time: a 3T diffusion tensor imaging study of mild traumatic brain injury. *AJNR American journal of neuroradiology* (2008) 29(5):967-73. Epub 2008/02/15. doi: 10.3174/ajnr.A0970. PubMed PMID: 18272556.
28. Freedman DJ, Ibos G. An Integrative Framework for Sensory, Motor, and Cognitive Functions of the Posterior Parietal Cortex. *Neuron* (2018) 97(6):1219-34. Epub 2018/03/24. doi: 10.1016/j.neuron.2018.01.044. PubMed PMID: 29566792; PubMed Central PMCID: PMCPMC5884170.
29. Galletti C, Kutz DF, Gamberini M, Breveglieri R, Fattori P. Role of the medial parieto-occipital cortex in the control of reaching and grasping movements. *Experimental brain research* (2003) 153(2):158-70. Epub 2003/10/01. doi: 10.1007/s00221-003-1589-z. PubMed PMID: 14517595.
30. MacKinnon CD. Sensorimotor anatomy of gait, balance, and falls. *Handbook of clinical neurology* (2018) 159:3-26. Epub 2018/11/30. doi: 10.1016/b978-0-444-63916-5.00001-x. PubMed PMID: 30482322; PubMed Central PMCID: PMCPMC7069605.

9 Figures

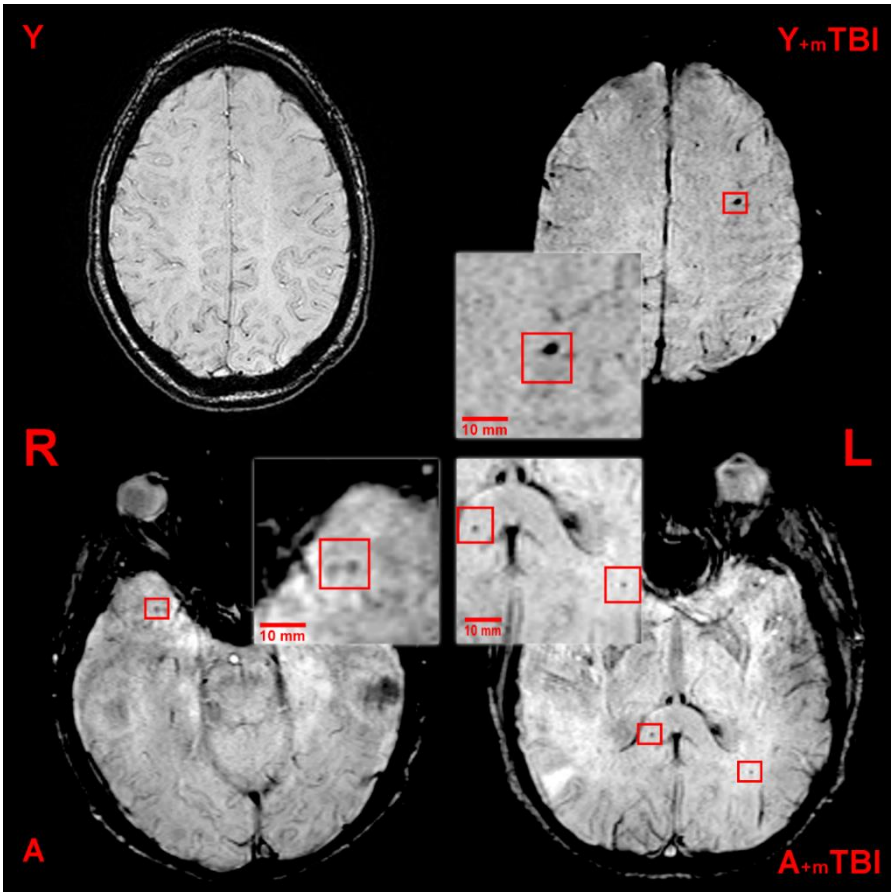

Figure 1. Axial susceptibility weighted (SWI) magnetic resonance images (MRI, 3 Tesla) of a young control patient (Y, 38-year-old, male), a young patient following mild traumatic brain injury (Y+mTBI, 36-year-old male GCS:15), an aged control patient (A, 67-year-old male) and an aged patient with mild TBI (A+mTBI, 65-year-old male, GCS:15). Cerebral microbleeds (CMB) appear as ovoid, hypointense lesions indicated by the red squares (R: right, L: left).

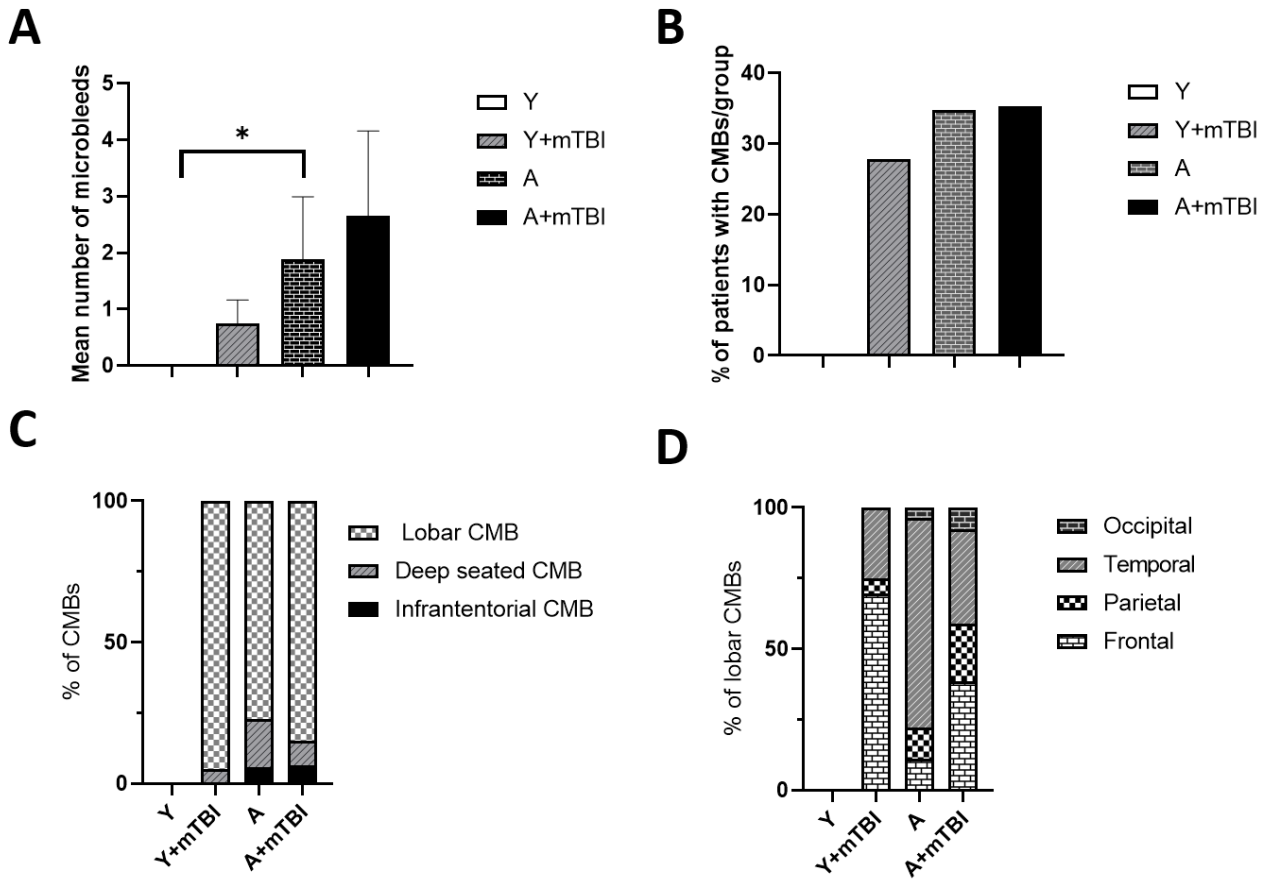

Figure 2. The effect of mild traumatic brain injury on the development and characteristics of cerebral microbleeds in the elderly. A: Mean number of cerebral microbleeds (CMB) in young control (Y) patients (n=20, age: 25,09 +/- 5,63 years), young patients after mild traumatic brain injury (Y+mTBI) (n=17, age: 24,65 +/- 10,22 years), aged control patients (A) (n=23, age: 68,36 +/-4,88 years) and aged patients with mTBI (A+mTBI, n=17, age: 71,86 +/- 7,31 years). Data are mean ± S.E.M., *P<0.05 vs. YC, ns: non-significant. **B**: Number of patients with CMBs in the studied groups is expressed as per cent of total number or patients in each group (young control (Y) patients (n=20, age: 25,09 +/- 5,63years), young patients after mild traumatic brain injury (Y+mTBI) (n=17, age: 24,65 +/- 10,22 years), aged control patients (A) (n=23, age: 68,36 +/-4,88) and aged patients with mTBI (A+mTBI) (n=17, age: 71,86 +/- 7,31 years). *P<0.05 vs. YC. **C** depicts localization of CMBs in each group as number of lobar, deep seated (basal ganglion) and infratentorial CMBs expressed as per cent (%) of total number of CMBs. Note that the majority of CMBs can be found supratentorially (lobar

and basal ganglion), however a small number of microbleeds appears in infratentorial location in aged patient after mild traumatic brain injury (mTBI). The difference did not reach statistical significance. **D:** Lobar distribution of supratentorial CMBs in each studied group of patients (frontal, temporal, parietal, occipital). Please note that aging enhances the number of parietal and occipital CMBs after 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).

(Y): n=20, 10 females, 10 males, age: 25,09 +/- 5,63 years; young + mTBI (Y+mTBI): n=17, 11 females, 6 males, age: 24,65 +/- 10,22 years ; aged (A): n=23, 16 females, 7 males, age: 68,36 +/-4,88 years; aged + mTBI (A+mTBI): n=17, 9 females, 8 males, age: 71,86 +/- 7,31 years..

Table 1. General description and main cardiovascular comorbidities of the study groups.

Group	Age (Mean+/- SD)	Sex		Hypertension		Smoking		Urea		Creatinine		Total Cholesterol		Low Density Lipoprotein	
		Female	Male	Yes	No	Yes	No	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Young control (Y)	25,09 +/- 5,63	50%	50%	10,0%	90,0%	5,0%	95,0%	85,0%	15,0%	85,0%	15,0%	90,0%	10,0%	95,0%	5,0%
Young trauma (Y+mTBI)	24,65 +/- 10,22	61,1%	35,3%	5,88%	94,12%	0%	100%	88,24%	11,76%	76,47%	26,53%	94,12%	5,88%	100,0%	0%
Aged control (A)	68,36 +/- 4,88	69,6%	30,4%	60,87%	39,13%	4,35%	95,65%	91,3%	8,7%	91,3%	8,7%	56,52%	48,43%	78,26%	21,74%
Aged trauma (A+mTBI)	71,86 +/- 7,31	52,9%	47,1%	88,24%	11,76%	17,65%	82,35%	82,35%	17,65%	52,94%	47,06%	82,35%	17,65%	100,0%	0%