Research summary

The summary is organized according to the original aims.

Aim 1, 2 and 3 (1.:Are hemorrhagic, or non-hemorrhagic focal traumatic lesions associated with the more severe axonal damage? 2.: Which lesion parameters indicate the more severe axonal damage? 3.: Proposal of a simple trauma MRI lesion classification):

Corresponding to these points we published the paper entitled „Both hemorrhagic and non-hemorrhagic traumatic MRI lesions are associated with the microstructural damage of the normal appearing white matter" (Behavioural Brain Research, Q1, IF = 3.1, PMID: 28249729).

The main finding of this study was that both hemorrhagic and non-hemorrhagic focal traumatic lesions indicate global white matter damage (defined by diffusion tensor imaging, DTI), however, regarding hemorrhagic lesions only if they are located in deep brain regions (basal ganglia and brainstem). The significance of these findings is that focal traumatic lesions can be readily detected under routine clinical circumstances, unlike DTI which can be regarded as the „gold standard" tool for non-invasive assessment of axonal damage, but to date requires complicated post-processing and generally group wise statistics.

As a simple traumatic lesion classification system suggestive for global white matter damage, we suggest the following: For non-hemorrhagic lesions, using the originally histology based Adams-Gentry (Gentry LR. Imaging of closed head injury. (1994) Radiology. 191 (1): 1-17.) classification appears to be feasible (grade I = subcortical lesions, grade II = corpus callosum lesions, Grade III = basal ganglia lesions, Grade IV = brainstem lesions), as even a single subcortical non-hemorrhagic lesion was associated with axonal damage in our study. For hemorrhagic lesions (i.e. microbleeds), however, an even more simple grading can be used: grade 0 = no microhemorrhages or microhemorrhages present not deeper than the corpus callosum; grade 1 = microhemorrhages located at the basal ganglia and/or brainstem. The finding that subcortical and corpus callosum microbleeds did not indicate white matter damage is somewhat surprising. The most probable explanation is that in these regions microvascular vulnerability may be higher than axonal vulnerability.

Below we inserted the abstract of this study, for further details please see the publication.

Traumatic microbleeds (TMBs) and non-hemorrhagic lesions (NHLs) on MRI are regarded as surrogate markers of diffuse axonal injury. However, the actual relation between lesional and diffuse pathology remained unclear, since lesions were related to clinical parameters, largely influenced by extracranial factors. The aim of this study is to directly compare TMBs, NHLs and their regional features with the co-existing diffuse injury of the normal appearing white matter (NAWM) as measured by diffusion tensor imaging (DTI). Thirty-eight adults with a closed traumatic brain injury (12 mild, 4 moderate and 22 severe) who underwent susceptibility weighted imaging (SWI), T1-, T2 weighted and FLAIR MRI and routine CT were included in the study. TMB (on SWI) and NHL (on T1-, T2 weighted and FLAIR images) features and Rotterdam

scores were evaluated. DTI metrics such as fractional anisotropy (FA) and mean diffusivity (MD) were measured over different NAWM regions. Clinical parameters including age; Glasgow Coma Scale; Rotterdam score; TMB and NHL features were correlated to regional NAWM diffusivity using multiple regression. Overall NHL presence and basal ganglia area TMB load were significantly, negatively correlated with the subcortical NAWM FA values (partial r=-0.37 and -0.36; p=0.006 and 0.025, respectively). The presence of any NHL, or TMBs located in the basal ganglia area indicates diffuse NAWM damage even after adjusting for clinical and CT parameters. To estimate DAI, a conventional lesional MRI pathology evaluation might at least in part substitute the use of quantitative DTI, which is yet not widely feasible in a clinical setting.

Aim 4: Is the proposed MRI lesion classification predictive of clinical outcome?

Unfortunatley, it was not possible to answer this question because of the low adherence of the patients for returning to the follow-up cognitive and clinical tests.

Instead of this, we raised a new research aim: Are the DTI parameters of the white matter closely surrounding microbleeds abnormal? The rationale for this question is that microbleeds were traditionally regarded as surrogate markers for axonal injury, however our previous findings and also more recent peer publications supported that subcortical/corpus callosum microbleeds are not necessarily linked to global axonal damage. But associations with perilesional axonal damage remained unclear.

To conduct this study we recruited both non-traumatic patients with microbleeds and both healthy controls that were not part of the original work-plan. This happened in the extension year (4th year) of the research project, after pandemic lockdown. The manuscript regarding this study has been completed but is to be published.

Below we provide a brief summary of this study:

Methods:

24 patients (15 males) with definitive traumatic microbleeds in SWI were selected out of overall 91 traumatic brain injury patients. Further 24 age and sex matched healthy control subjects were enrolled. As a third group, 18 age and sex matched patients with non-traumatic cerebral microbleeds were included. All patients underwent an identical MRI protocol including high resolution 3D T1 weighted imaging, SWI and DTI. All patients gave informed consent and the study was approved by the local ethical committee.

Using FSL (FMRIB, Oxford) environment, a pipeline was developed to measure DTI parameters (fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity) in a 4 and also a 6 mm diameter spheroid shell shaped region of interest (ROI) around microbleeds, excluding the inner region (2 mm exclusion for 4 mm diameter ROIs, and 4 mm exclusion for 6 mm diameter ROIs) possibly affected by T2* and phase alteration due to microbleeds. These ROIs were mirrored to the contralateral side also using FSL tools and manual correction if necessary based on anatomy. Such ROIs were applied for microbleeds in both the traumatic and the non-

traumatic groups (subcortical and corpus callosum regions), for maximum three microbleeds per patient (if more than three microbleeds were present in a patient, the three one located the most centrally in the white matter were selected). The ROIs (and corresponding contralateral ROIs) from the traumatic group were translated to the healthy control group. This resulted in 48 ROI pairs in the traumatic and healthy control group, and 39 ROI pairs in the non-traumatic group. Normal distribution was assessed using the Shapiro Wilk test. Ipsilateral and contralateral ROI DTI parameters among all groups were compared using Kruskal-Wallis (with Conover post hoc) test. P values under 0.05 were considered significant.

Results:

Within all groups, regarding the 6 mm diameter ROIs, no significant difference in DTI parameters were found between lesion and contralateral side ROIs. Regarding 4 mm diameter ROIs, both in the traumatic and in the non-traumatic groups, lesion side fractional anisotropy was significantly reduced, while radial diffusivity was significantly increased compared to the contralateral side.

Discussion:

Peri-microbleed white matter seems not to be damaged based on DTI parameters when compared to the contralateral side in traumatic brain injury. This suggests that vascular injury does not co-localize with axonal injury which is in line with a recent study including histopathological analysis (PMID: 31608359). The diffusion parameter alterations seen in the very adjacent (4 mm diameter) peri-lesional white matter appeared to be non-specific to traumatic microbleeds, as they occurred around non-traumatic microbleeds as well. This may be explained by either a paramagnetic artifact caused by hemosiderin, or a common neurotoxic effect of iron deposits.

The overall suggestion regarding the significance of traumatic microbleeds based on our studies is that microbleeds in general are not reliable markers of axonal injury, they should be rather recognized as a separate component of traumatic brain injury potentially with reginal neurotoxic effects. However, for their formation at deep brain regions that are known to be mechanically more fixed, higher traumatic energy is needed, which also results in diffuse axonal damage.

Aim 5: What is the optimal SWI acquisition timepoint?

To answer this question, a rat microbleed MR model was established which showed that on SWI microbleeds become invisible at 24 hours after injury, but reappear after the second day (but in fact are continuously present based on histology). (Journal of Neurotrauma, Q1, IF = 4.33, PMID: 30421664)

Below we inserted the abstract of this study, for further details please see the publication.

Previously, we reported human traumatic brain injury cases demonstrating acute to subacute microbleed appearance changes in susceptibility-weighted imaging (SWI-magnetic resonance imaging [MRI]). This study aims to confirm and characterize such temporal microbleed appearance alterations in an experimental model. To elicit microbleed formation, brains of male Sprague Dawley rats were pierced in a depth of 4 mm, in a parasagittal position bilaterally using 159 µm and 474 µm needles, without the injection of autologous blood or any agent. Rats underwent 4.7 T MRI immediately, then at multiple time points until 125 h. Volumes of hypointensities consistent with microbleeds in SWI were measured using an intensity threshold-based approach. Microbleed volumes across time points were compared using repeated measures analysis of variance. Microbleeds were assessed by Prussian blue histology at different time points. Hypointensity volumes referring to microbleeds were significantly decreased (corrected $p < 0.05$) at 24 h compared with the immediate or the 125 h time points. By visual inspection, microbleeds were similarly detectable at the immediate and 125 h imaging but were decreased in extent or completely absent at 24 h or 48 h. Histology confirmed the presence of microbleeds at all time points and in all animals. This study confirmed a general temporary reduction in visibility of microbleeds in the acute phase in SWI. Such short-term appearance dynamics of microbleeds should be considered when using SWI as a diagnostic tool for microbleeds in traumatic brain injury and various diseases.

Aim 6: (Additional aim, not included in original work plan)

As the number of traumatic brain injury patients who underwent MRI rose, we realized that it is possible to check if the phenomenon of temporary microbleed invisibility exists in humans, using a retrospective analysis. This study showed that microbleeds are present less frequently from 24h to 72h after trauma, most likely because the phenomenon of temporal microbleed invisibility exists in humans as well. (Frontiers Neuroscience, Q2, IF = 3.57, PMID: 34658762)

The practical consequence of this and the previous studies is that improper timing of SWI may lead to false-negative results regarding microbleeds.

Below we inserted the abstract of this study, for further details please see the publication.

Purpose: A former rodent study showed that cerebral traumatic microbleeds (TMBs) may temporarily become invisible shortly after injury when detected by susceptibility weighted imaging (SWI). The present study aims to validate this phenomenon in human SWI. Methods: In this retrospective study, 46 traumatic brain injury (TBI) patients in various forms of severity were included and willingly complied with our strict selection criteria. Clinical parameters potentially affecting TMB count, Rotterdam and Marshall CT score, Mayo Clinic Classification, contusion number, and total volume were registered. The precise time between trauma and MRI [5 h 19 min to 141 h 54 min, including SWI and fluid-attenuated inversion recovery (FLAIR)] was individually recorded; TMB and FLAIR lesion counts were assessed. Four groups were created based on elapsed time between the trauma and MRI: 0-24, 24-48, 48-72, and >72 h. Kruskal-Wallis, ANOVA, Chi-square, and Fisher's exact tests were used to reveal differences among the groups within clinical and imaging parameters; statistical power was calculated retrospectively for each comparison. Results: The Kruskal-Wallis ANOVA with

Conover post hoc analysis showed significant ($p = 0.01$; $1-\beta > 0.9$) median TMB number differences in the subacute period: 0-24 h = 4.00 (n = 11); 24-48 h = 1 (n = 14); 48-72 h = 1 (n = 11); and 72 h ≤ 7.5 (n = 10). Neither clinical parameters nor FLAIR lesions depicted significant differences among the groups. Conclusion: Our results demonstrate that TMBs on SWI MRI may temporarily become less detectable at 24-72 h following TBI.

Aim 7: (Additional aim, not included in original work plan)

The cause of the phenomenon of temporal microbleed invisibility remained unclear. To test if antiplatelet or anticoagulation therapy might affect the phenomenon, we repeated the rat microbleed MRI model on groups of rats given ASA and enoxaparin.

This work has been completed, but has yet to be published.

Please find a brief summary below.

Previously, we reported a rat study which showed that microbleeds became less visible or even invisible in susceptibility-weighted imaging (SWI – magnetic resonance imaging [MRI]). This study aims to reveal the effects of commonly used pharmaceutical agents such as acetylsalicylic acid (ASA) and enoxaparin on this phenomenon. We examined three groups of male Sprague-Dawley rats, each group consisted of ten rats. The first group received 5 mg/kg ASA, the second group received 5 mg/kg enoxaparin and the third group, the control group, received 1 ml 0.9% NaCl solution. Treatment was given subcutaneously. The first treatment was given four days before inducing microbleeding and repeated at every 24 th hour until the injury procedure. To induce microbleeding formation, brains of the rats were pierced in a depth of 4 mm, in a parasagittal position bilaterally using 159 µm and 474 µm needles, without the injection of autologous blood or any agent. Rats underwent 4.7 T MRI immediately, 24 hours, 48 hours and 96 hours after the procedure. Volumes of hypointensities consistent with microbleeds in SWI were measured using an intensity threshold-based approach. Microbleed volumes across time points were compared using repeated measures analysis of variance. Hypointensity volumes referring to microbleeds were significantly decreased (corrected $p < 0.05$) at 24 h compared with the immediate or the 96 h time points in all groups. There was no significant difference in the decrease of the hypointensity volumes between the control and the other two groups. Our study proved that neither ASA nor enoxaparin affect the „disappearance" of the microbleeds in acute phase SWI.

Aim 8.: (Additional aim, not included in original work plan)

Our database let us test if aging interacts with traumatic microbleed formation. The lobar distribution of supratentorial microbleeds showed that aging enhances the formation of parietal and occipital microbleeds after traumatic brain injury. (Frontiers Aging Neuroscience, Q1, IF = 5,7, PMID: 34658836)

Below we inserted the abstract of this study, for further details please see the publication.

A traumatic brain injury (TBI) induces the formation of cerebral microbleeds (CMBs), which are associated with cognitive impairments, psychiatric disorders, and gait dysfunctions in patients. Elderly people frequently suffer TBIs, especially mild brain trauma (mTBI). Interestingly, aging is also an independent risk factor for the development of CMBs. However, how TBI and aging may interact to promote the development of CMBs is not well established. In order to test the hypothesis that an mTBI exacerbates the development of CMBs in the elderly, we compared the number and cerebral distribution of CMBs and assessed them by analysing susceptibility weighted (SW) MRI in young (25 ± 10 years old, n = 18) and elder (72 ± 7 years old, n = 17) patients after an mTBI and in age-matched healthy subjects (young: 25 ± 6 years old, n = 20; aged: 68 ± 5 years old, n = 23). We found significantly more CMBs in elder patients after an mTBI compared with young patients; however, we did not observe a significant difference in the number of cerebral microhemorrhages between aged and aged patients with mTBI. The majority of CMBs were found supratentorially (lobar and basal ganglion). The lobar distribution of supratentorial CMBs showed that aging enhances the formation of parietal and occipital CMBs after mTBIs. This suggests that aging and mTBIs do not synergize in the induction of the development of CMBs, and that the different distribution of mTBI-induced CMBs in aged patients may lead to specific age-related clinical characteristics of mTBIs.

International scientific meeting posters/presentations related to the project:

International Society for Magnetic Resonance in Medicine (ISMRM) Conference, Honolulu, HI, USA 2017

International Society for Magnetic Resonance in Medicine (ISMRM) Conference, Paris 2018

European Congress of Radiology, Vienna 2018- DOI:10.1594/ecr2018/C-2819 Best of Subspeciality Award –European Society for Hybrid, Molecular and Translational Imaging

European Congress of Radiology, Vienna 2020 DOI:10.26044/ecr2020/C-10087

12th Slovenian- Croatian-Hungarian-Slovakian Radiological Symposium- Ragaska Slatina

7th Pannonian Symposium on CNS Injury, Pécs, Hungary 2017