Detailed report for „Translational MRI research of mild traumatic brain injury" (OTKA/109132, 2013-2016)

The report below is structured based on the aims listed in points in the original detailed research plan.

Aims 1. and 3. -MRI volumetric studies (2013-2015)

When getting to the first pilot analyses, to check if there might be a difference in the yielded results, we compared the two most widely applied volume analysis strategies, the automated volumetric analysis and the voxel-based morphometry (VBM). It was also a question, if the different head size correction algorithms enabling inter-subject comparisons (general linear model (GLM) based, or proportion based) may lead to different results. Surprisingly, regarding certain comparisons, these methods led to meaningfully different results.

To more generally test and publish this methodological issue, we focused on the very widely studied question of gender differences between brain structures volumes. T1-weighted MR images were collected in 99 healthy, Caucasian, university students (66 female subjects; mean age: 23.1 ± 2.3, range: 19-31 years). Sexual dimorphism in hippocampus was investigated by automated MRI volumetry and VBM using both GLM and proportion head-size correction strategies. Absolute hippocampal volumes were larger in men than women. After adjusting for head-size, the proportion method indicated larger hippocampi in women than men, while no gender differences were found using the GLM approach. Investigating absolute hippocampal volumes in 15 head-size matched pairs of males and females indicated no gender differences. We suggested that there is no sexual dimorphism in hippocampal size and the apparent gender differences found by the proportion method may have more to do with head-size than with sex. We concluded that GLM and proportion head-size correction strategies are not interchangeable and may yield different results.

The importance of these findings is mostly related to scientific reproducibility across MRI volumetry or VBM studies, and perhaps will therefore aid the proper choice of volumetric analysis strategy for further studies. These findings were published, for more details please see our publication list: "Are there any gender differences in the hippocampus volume after head-size correction? A volumetric and voxel-based morphometric study."

Our initial analyses related to TBI suggested that the injury induced brain volume changes may be biased by diurnal volume changes of the brain (such diurnal brain volume fluctuations have been observed and published recently by an other research group: Neuroimage. 2015 Sep;118:126-32. doi: 10.1016). Along with the limited time-of-day MRI availability, this highly restricted the number of the reasonably includible patients. In order to investigate more robust volume changes, we extended our volumetric studies on severe TBI, and to allow a wider feasibility, CT volumetric analyses was introduced. In these

studies, we primarily concentrated on post-traumatic hydrocephalus and edema measured by lateral ventricle volumes that were related to other CT findings, clinical and biomarker data, in a follow-up setting. These studies were performed in a collaboration with the University of Florida TBI research group:

We hypothesized that lateral ventricular volume (LVV) asymmetry is an earlier sign of developing asymmetric intracranial pathology than midline shift. An analysis was performed on data from 84 adults with blunt sTBI requiring a ventriculostomy who presented to a Level I trauma center. Seventy-six patients underwent serial CTs within 3 h and an average of three scans within the first 10 d of sTBI. Left and right LVVs were quantified by computer-assisted manual volumetric measurements. LVV ratios (LVR) were determined on the admission CT to evaluate ventricular asymmetry. As a control group, 76 age and sex matched patients with a non-traumatic indication were retrospectively included. The relationship between the admission LVR value and subsequent midline shift development in the TBI groups was tested using receiver operating characteristic (ROC) analysis, and odds ratio (OR) and relative risk tests. Sixty patients had no >5 mm midline shift on the initial admission scan. Of these, 15 patients developed it subsequently (16 patients already had >5 mm midline shift on admission scans). For >5 mm midline shift development, admission LVR of >1.67 was shown to have a sensitivity of 73.3% and a specificity of 73.3% (area under the curve=0.782; $p<0.0001$). LVR of >1.67 as exposure yielded an OR of 7.56 ($p<0.01$), and a risk ratio of 4.42 ($p<0.01$) for midline shift development as unfavorable outcome. We proposed that LVR captures LVV asymmetry and is not only related to, but also predicts the development of midline shift already at admission CT examination. We determined the normal lateral ventricle volume asymmetry in the control group, average LVR was 1.14 (SD=0.11), median was 1.11 (min. LVR=1.00, max. LVR=1.44), which allowed the LVR value of >1.67 to be used as a threshold to define pathological asymmetry. For illustration, see figures 1 and 2.

We concluded that lateral ventricles may have a higher "compliance" than midline structures to developing asymmetric brain pathology. LVR analysis is simple, rapidly accomplished and may allow earlier interventions to attenuate midline shift and potentially improve ultimate outcomes. For more details, please find these results in our publication list: “Lateral Ventricle Volume Asymmetry Predicts Midline Shift in Severe Traumatic Brain Injury”.

Our further findings related to these studies were yet published at leading scientific meetings:

Conventional vs. quantitative approach in assessing post-traumatic ventriculomegaly and its relation to 6-month outcomes in severe traumatic brain injury:

Subjective assessments leave the relationship of ventriculomegaly and outcome measures elusive. We hypothesized that ventricular volumetric based identification of ventriculomegaly could add to subjective radiologic assessment of ventriculomegaly in predicting outcome. A retrospective analysis was performed on 84 adults with blunt sTBI requiring a ventriculostomy presenting to a Level I Trauma Center. Serial CTs within 3 hrs and an average of 3 additional scans within the first 10 days of sTBI and 6-month outcome data, Disability Rating Scale (DRS) and Glasgow Outcome Scale-Extended (GOS-E), were available for 64 patients. Subjective assessment for ventriculomegaly and quantified ventricular volumes

were determined. Relative enlargement to initial volume was calculated. The relationship to 6-month outcome was assessed between patients without ventricular enlargement and each of the following groups: (A) Subjective radiological diagnosis of ventriculomegaly. (B through E) Quantitative measurement groups: (B) any, (C) overall, (D) bilateral, (E) unilateral enlargement. Mann-Whitney U-test determined significance ($p < 0.05$). Ten surviving patients with unilateral enlargement (E) had significantly worse DRS and GOS-E scores than all other 19 surviving patients (mean scores for group E: DRS = 10, GOS-E = 4; for nonunilateral enlargement group: DRS = 4, GOS-E = 6). Other groups (AD) did not differ in outcome from non-enlargement patients. Quantitative approach identified a subgroup of sTBI patients with unilateral ventricle enlargement indicating poor outcome while other types or subjective-diagnosed ventricle enlargement did not.

Lateral ventricle volume asymmetry is related to spectrin breakdown:

Biomarkers of neuronal injury together with neuroimaging could be used to better evaluate sTBI severity. The relationship between early lateral ventricle volume asymmetry, Rotterdam score, Marshall CT Classification and cerebrospinal fluid (CSF) biomarkers were assessed. This retrospective study included 84 adults with blunt sTBI requiring ventriculostomy presenting to a Level I Trauma Center. 64 patients had an initial CT done within 3 hrs after TBI and quantitative CSF biomarker data (UHCL-1, SBDP145, SBDP150, SBDP120, MAP2, MBP, S100B) available within 24 hrs after injury. Lateral ventricle volumes were quantified by computer-assisted manual volumetric measurements and their ratio (LVR) was calculated to capture ventricular asymmetry. Marshall and Rotterdam scores also were determined. Non-parametric tests were used to assess the correlations. LVR values were significantly correlated with the Rotterdam score ($rho = 0.45$, $p < 0.001$) and Marshall CT classification ($rho = 0.29$, $P = 0.008$). There were 68 patients who had both LVR and biomarker levels available. The only biomarker that was significantly associated with LVR was SBDP145 ($rho = 0.30$, $p = 0.023$). In those with LVR< = 1.8, mean SBDP145 values taken at the earliest time point within 24 hours was 64.6 (SD97.6) and in those with LVR > 1.8 was 103.0 (SD 81.4) ($p = 0.02$). SBDP145 was also correlated with the Rotterdam score ($rho = 0.38$, $p < 0.001$) and with Marshall Classification ($rho = 0.23$, $p = 0.017$). Our results suggest that asymmetric distortion is an important component of brain pathologies in sTBI and is associated with both Rotterdam and Marshall Classifications. These CT findings are associated with early elevations of SBDP145, suggesting SBDP145 is an important indicator of sTBI severity.

Aim 2 -fMRI studies (2013-)

It was not possible to reproduce the original findings from a low population when achieving a relatively higher number of included patients. As an explanation, we supposed that the neurovascular response mechanisms might be generally affected but not related to specific tasks. To investigate general blood flow alterations, we started perfusion (arterial spin labeling, ASL) studies. Three mTBI patients and three healthy control subjects underwent ASL acquisition twice according to our original fMRI study set-up. Average perfusion rate difference between the cortex and white matter was larger in the mTBI patients acutely than a month later or than in the healthy subjects, which implies an acute neurovascular regulation disorder. However, a larger sample size was needed to test the statistical significance of this finding. Unfortunately ASL was only available at the 1.5 T MRI in the diagnostic center of Pécs, which does not meet the standards of international ASL studies, moreover our research group had no regular

research access to the 1.5 T system. Therefore we joined the research group of the diagnostic center of Pécs to apply for an ASL license for the 3T system. This process is still running.

Aim 4. and 5. -rat TBI studies (2014-2016)

The originally planned Marmarou weight drop model turned out to induce too heterogeneous brain pathologies that made the image analyses impossible, or, a very high number of traumatized animals should have been included to achieve the proper number of evaluable cases. Therefore we switched the animal TBI model to a very highly reproducible electricity induced trauma model.

Our purpose was to verify the following phenomenon in vivo using quantitative magnetic resonance imaging (MRI): Neuronal compression may occur following brain injuries in the cortex and hippocampus. As well being characterized by previous histological studies in rats, the majority of these neurons undergo hyperacute recovery rather than apoptotic death.

Twenty male Wistar rats were assigned into injured or sham-injured groups (n = 10). The injured group underwent an electric trauma model to provoke compacted neuron formation. A T1 map was acquired prior to the injury and 10 T1 maps were acquired consecutively over a period of 2.5 hours after the injury, using a 3.0T scanner. Voxelwise statistical analyses were performed between timepoints. To enable comparison with the histological appearance of the compacted neurons, silver staining was performed on a sham-injured rat and five injured rats, 10, 40, 90, 150, and 300 minutes after the injury.

A significant (corrected $p < 0.05$) increase in average T1 from the preinjury (895.24 msec) to the first postinjury timepoint (T1 = 951.37 msec) was followed by a significant (corrected $P < 0.05$) decrease (return) up to the last postinjury timepoint (T1 = 913.16 msec) in the voxels of the cortex and hippocampus. No significant (corrected $P < 0.05$) change in T1 was found in the sham-injured group.

The spatial and temporal linkages between the MRI T1 changes and the histological findings suggest that neuronal compaction and recovery is associated with T1 alterations. This association might be explained by inter-compartmental water movement. MRI therefore offers the possibility of in vivo investigations of neuronal compaction and recovery. Figures 3 and 4 illustrate these results.

Please find this study for more details in our publication list: “In vivo detection of hyperacute neuronal compaction and recovery by MRI following electric trauma in rats.”

Additional aims - Human susceptibility weighted imaging (SWI) TBI studies (2015-)

Due to the recent trends and the fact that this method might enable TBI assessment at an individual level (in contrast with group based statistics), susceptibility weighted imaging (SWI) was added to our MRI protocol. SWI is a very sensitive tool for the detection of microbleeds in TBI. The number and extent of such traumatic microbleeds (TMBs) have been shown to correlate with the severity of the injury and the clinical outcome. However, the acute dynamics of TMBs have not been revealed so far. We aimed to reveal possible SWI microbleed appearance changes in the acute phase.

We presented five closed TBI patients who underwent SWI very early (average=23.4 h), and once again a week (average=185.8 h) after the injury. The TMBs were mapped at both time points by a conventional radiological approach and their numbers and volumes were measured with manual tracing tools by two observers. TMB counts and extents were compared between time points.

TMBs were detected in four patients, three of them displaying an apparent TMB change. In these patients, TMB confluence and apparent growth were detected in the corpus callosum, coronal radiation or subcortical white matter, while unchanged TMBs were also present. These changes caused a decrease in the TMB count associated with an increase in the overall TMB volume over time.

We have found a compelling evidence that diffuse axonal injury-related microbleed development is not limited strictly to the moment of injury: the TMBs might expand in the acute phase of TBI. We concluded that the timing of SWI acquisition may be relevant for optimizing the prognostic utility of this imaging biomarker. Key images related to these findings are shown in figure 5.

For more details, please find this paper in our publication list: “Microbleeds may expand acutely after traumatic brain injury.”

Most recently we aimed to test how strictly the microvascular injury revealed by SWI is related to the actual diffuse axonal injury (DAI), as revealed by diffusion tensor imaging (DTI):

DAI is a substantial pathological component of traumatic brain injury. Focal hemorrhagic (traumatic microbleeds = TMBs) and non-hemorrhagic lesions (NHLs) seen on SWI, and routine MRI methods are regarded as surrogate markers of DAI.

The aim of this study was to directly compare TMBs, NHLs and their regional features with the microstructural integrity of the normal appearing white matter as measured by DTI, an advanced MRI tool regarded to be sensitive to DAI.

Thirty-eight adults with a closed traumatic brain injury (12 mild, 4 moderate and 22 severe) who underwent 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 CT Rotterdam score were evaluated. DTI metrics fractional anisotropy (FA) and mean diffusivity (MD) were measured by a region-of-interest approach over different brain regions. Clinical parameters including Glasgow Coma Scale; Rotterdam score; and MRI lesion features were correlated to regional diffusivity using multiple regression.

Overall NHL presence and basal ganglia area TMB load was significantly, negatively correlated with subcortical white matter and corona radiata FA values (partial r = -0.37 and -0.36; p=0.006 and 0.025, respectively).

Both overall non-hemorrhagic-, and hemorrhagic lesions of the basal ganglia area indicate widespread microstructural damage of the normal appearing white matter even after adjusting for clinical and CT parameters. The clinically feasible focal macroscopic pathology evaluation might substitute the use of DTI.

These findings were submitted to Behavioural Brain Research (Ref. No.: BBR-D-16-00741) and is being revised based on the editors and reviewers' comments. On acceptance, the expected publication is January 2017.

Figures

Figure 1. Admission and follow-up CT scan images of a representative patient with high admission LVR who subsequently developed significant midline shift. Midline shift was measured 1mm (not significant) on admission scan (<3 h post-injury) (left), while it has become 7mm (significant) on the follow-up scan at 20 h (right). The red line indicates the midline. External ventricular drainage is present on follow-up scan (hyperintense dot near right lateral ventricle).

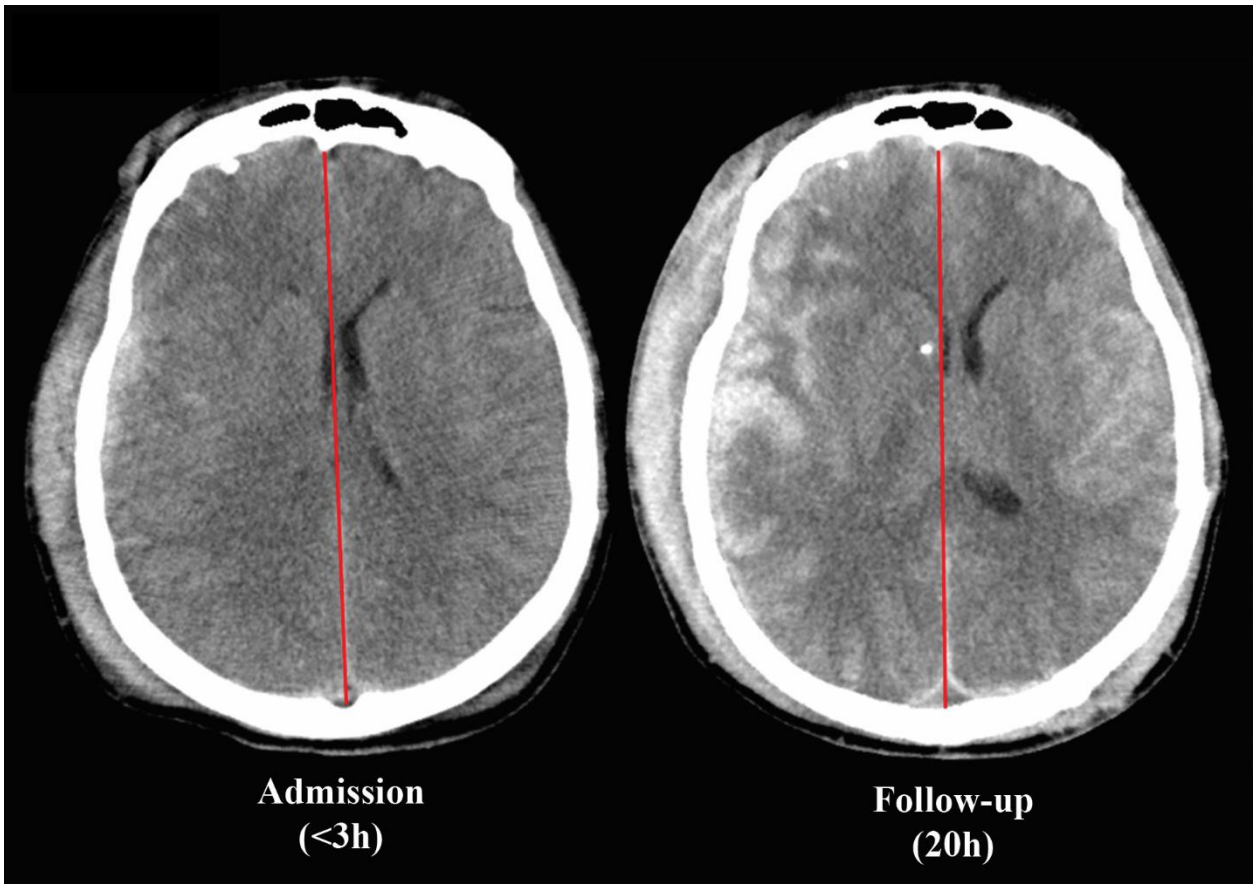

Figure 2. Concept of asymmetric intracranial pathology development.

A; 1. Normal brain 2. Initiation of asymmetric brain pathology (e.g. bleeding, edema) 3. Pathology propagation, ipsilateral ventricle compression causing lateral ventricular asymmetry 4. Further propagation causing midline shift.

B; 1. Normal brain 2. Initiation of ventricular entrapment 3. Pathology propagation, ventricle enlargement causing lateral ventricular asymmetry 4. Further propagation causing midline shift

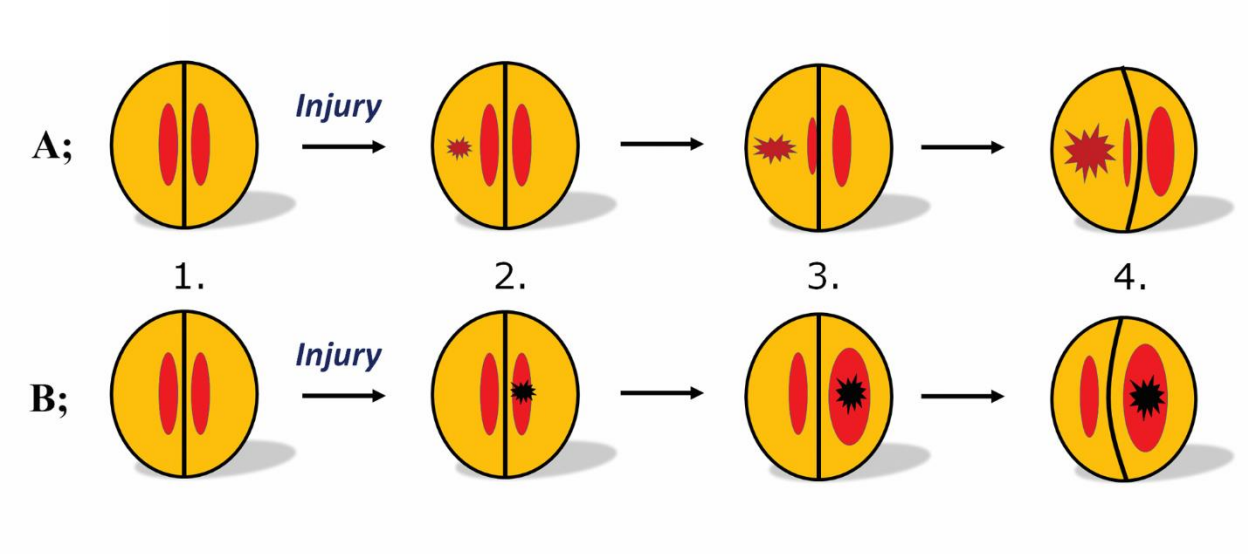

Figure 3. Yellow clusters depict voxels indicative of a significant (corrected $p < 0.05$) increase in T1 during the compacted neuron formation phase (between the pre-injured and first post-injured acquisition) and a significant (corrected $p < 0.05$) decrease in T1 during the compacted neuron recovery phase (between the first post-injured acquisition and the last post-injured acquisition)(=voxels of interest). The average T1 map of the animal group in the pre-injured state is used as a background image. Z = slice number. The red box shows slice Z = 7, the reference slice for Fig. 4. The image follows the radiological convention: the left side of the image shows the right side of the brain.

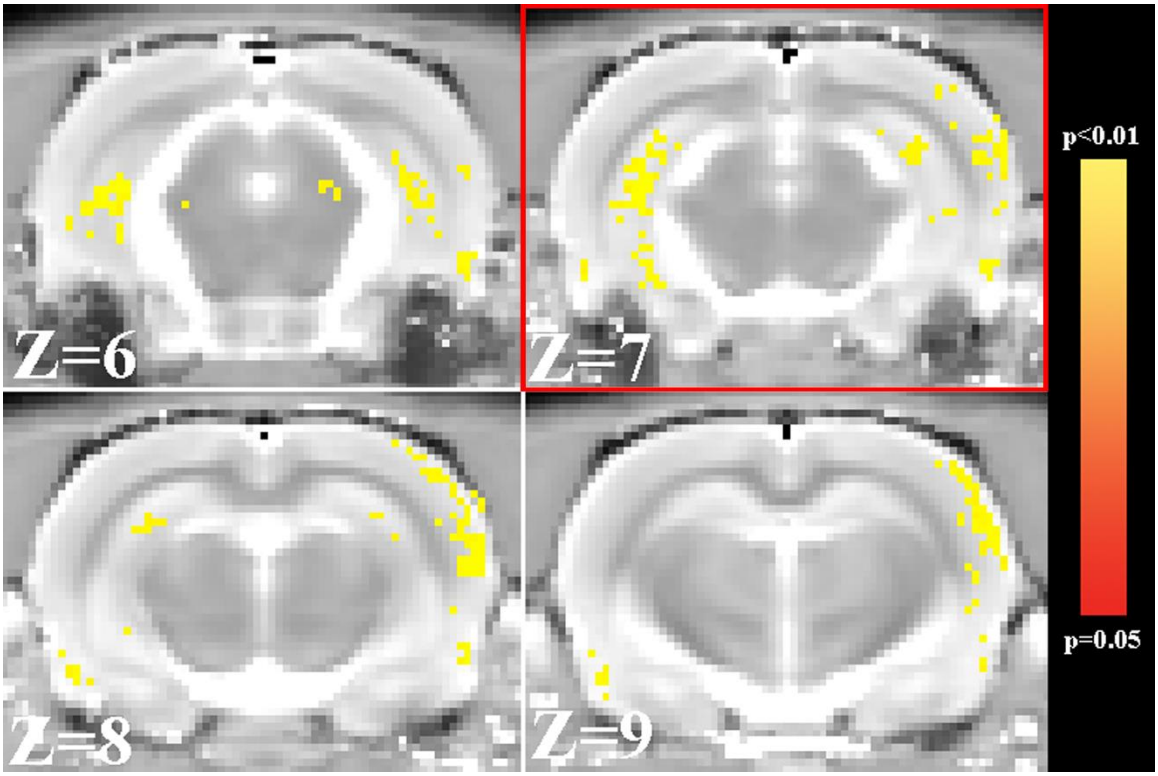

Figure 4. Typical compacted neuron distribution 10 min after the electric trauma (silver stain, overview image: 10x, inserts: 100x). The slice is matched to Fig. 3. Z = 7 slice (red box). The image follows the radiological convention: the left side of the image shows the right side of the brain. Yellow boxes indicate areas of compacted neuron formation. The box size illustrates the number of compacted neurons. Numbers show insert locations. The left cortical areas (4 and 8) and the right hippocampal – dental gyrus area (2) show massive compacted neuron formation. Milder compacted neuron formation can be seen in the left hippocampus – dental gyrus (3). The right cortical areas (1 and 5) are characterized by subtle compacted neuron formation. Compacted neurons are not present in the upper cortical and thalamic-mesencephalic regions (6 and 7).

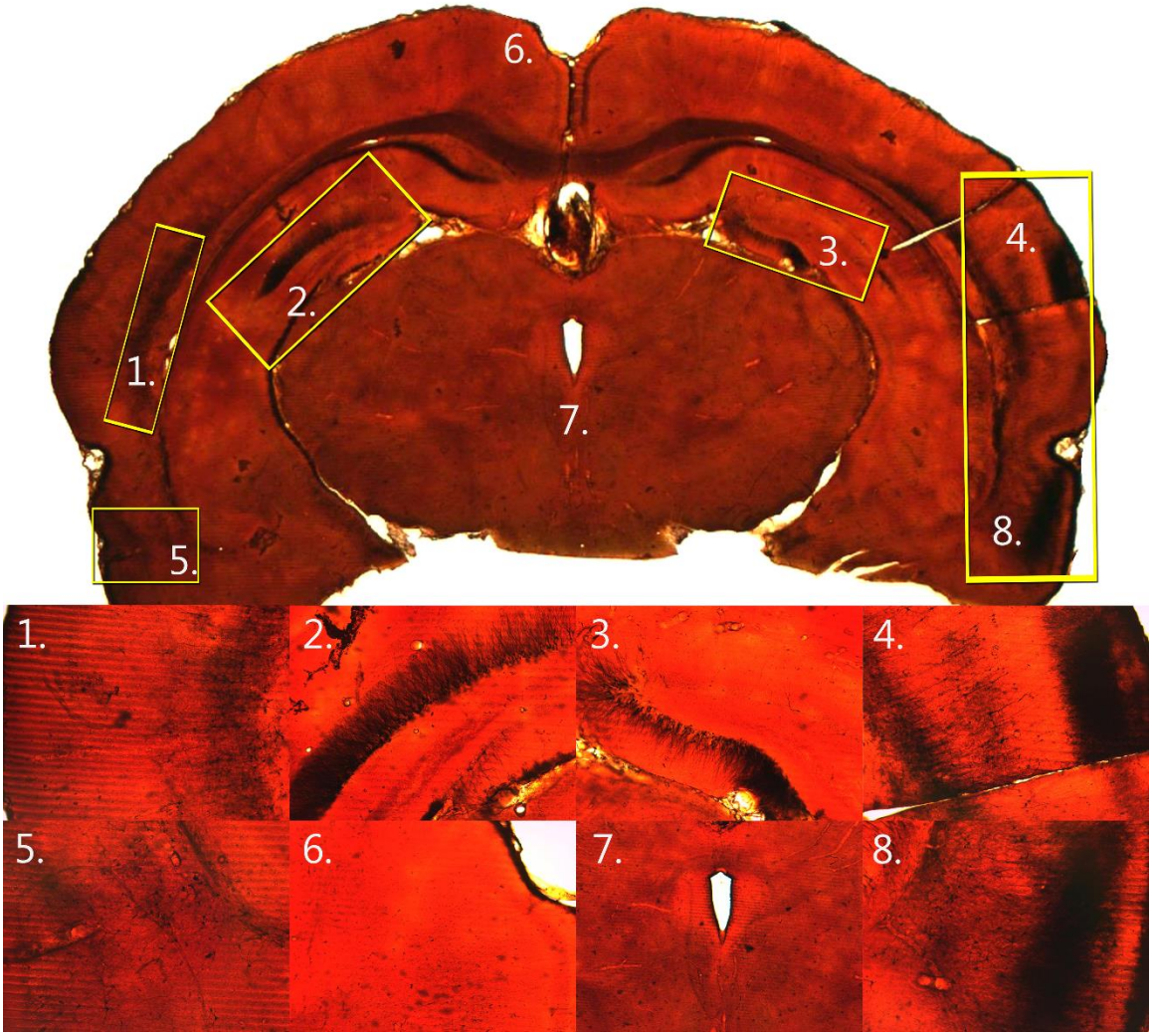

Figure 5. Traumatic microbleed growth and confluence example in the first week after TBI.

This figure shows the initial (< 24 h) and the follow-up (1 week) CT and SWI scans of a TBI patient. The inserts show identical anatomical locations in the co-registered initial and follow-up SWI scans, and the possible closest anatomical slice in the CTs and non-registered follow-up SWI scans per patient. Multiple TMBs can be seen in the left side of the splenium of the corpus callosum which underwent growth and confluence (has become one larger TMB on follow-up SWI). These microbleeds and changes are not detectable in the admission or follow-up CT scans either.