Original research article

Central nervous system microbleeds in the acute phase are associated with structural integrity by DTI one year after mild traumatic brain injury: A longitudinal study

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ABSTRACT

Introduction: Several imaging modalities are under investigation to unravel the pathophysiological mystery of delayed performance deficits in patients after mild traumatic brain injury (mTBI). Although both imaging and neuropsychological studies have been conducted, only few data on longitudinal correlations of diffusion tensor imaging (DTI), susceptibility weighted imaging (SWI) and extensive neuropsychological testing exist.
Methods: MRI with T1- and T2-weighted, SWI and DTI sequences at baseline and 12 months of 30 mTBI patients were compared with 20 healthy controls. Multiparametric assessment included neuropsychological testing of cognitive performance and post-concussion syndrome (PCS) at baseline, 3 and 12 months post-injury. Data analysis encompassed assessment of cerebral microbleeds (Mb) in SWI, tract-based spatial statistics (TBSS) and voxel-based morphometry (VBM) of DTI (VBM-DTI). Imaging markers were correlated with neuropsychological testing to evaluate sensitivity to cognitive performance and post-concussive symptoms.

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Results: Patients with Mb in SWI in the acute phase showed worse performance in several cognitive tests at baseline and in the follow-ups during the chronic phase and higher symptom severity in the post concussion symptom scale (PCSS) at twelve months post-injury. In the acute phase there was no statistical difference in structural integrity as measured with DTI between mTBI patients and healthy controls. At twelve months post-injury, loss of structural integrity in mTBI patients was found in nearly all DTI indices compared to healthy controls.

Conclusions: Presence of Mb detected by SWI was associated with worse cognitive outcome and persistent PCS in mTBI patients, while DTI did not prove to predict neuropsychological outcome in the acute phase.

1. Introduction

Mild traumatic brain injury (mTBI) affects approximately 100–300 per 100,000 individuals per year and is thereby one of the most common neurological disorders diagnosed in the emergency department [1]. While most patients with mTBI recover within weeks to months, a subgroup of 20–50% continues to complain about emotional, cognitive and/or somatic symptoms, also known as post-concussion syndrome (PCS) [2–5], whereas after one year 10% of mTBI patients still have symptoms [6,7].

Although overall test-results show normal cognitive performance several months after injury, some patients still complain of cognitive symptoms [8]. Even after decades of mTBI research, there is an ongoing debate about the exact pathophysiology of persistent neurobehavioral and cognitive symptoms [9]. A possible explanation is that they are the consequence of diffuse axonal injury (DAI) [10,11]. Current research projects focus on imaging signs to contribute to the diagnosis, prognosis and understanding of the pathomechanisms of mTBI [12]. Presence of individual pathoanatomical features on T1- or T2-weighted MR sequences, such as brain contusions, microbleeds (Mb) and foci of hemorrhagic axonal injury do not always correlate with poor outcome after mTBI [13–15]. Diagnostic strength of newer imaging modalities for PCS have been studied comparing cognitive status with DTI findings [16–25]. Quantitative DTI metrics, such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD), representing microstructural white matter lesions, seem to correlate with cognitive deficits during the first months after mTBI [19,20,23–25]. However, most studies have focused only on the acute or subacute phase [24,26–28]. Therefore, we evaluated structural integrity by means of T1- and T2-weighted, SWI and DTI MR sequences and PCS in the acute and chronic phase for its ability to predict cognitive performance and hypothesized that PCS and DTI abnormalities would correlate more than SWI abnormalities.

2. Methods

The full sample of the present study consisted of an mTBI group and a control group (CTRL). The mTBI group included consecutive patients presenting to the emergency department between August 2012 and December 2013. This cohort was part of a larger study, investigating the effect of 3-day sick leave vs. 7-day sick leave as described in Studerus-Germann et al. [29] mTBI was defined as an initial GCS of 13–15, loss of consciousness (LOC) lasting less than 30 min, posttraumatic amnesia (PTA) of less than 24 h and/or any alteration in mental status at the time of injury (e.g. feeling initially confused, dazed or disoriented). Inclusion criteria were as follows: Isolated mTBI without focal neurological deficits as defined above, CT without pathological findings, age at inclusion 18–64 years and German speaking. Exclusion criteria were: patients under the influence of alcohol (above 0.5 per mill blood alcohol), regular drug consumption, psychiatric disease under medical treatment (at present or in the last two years), previously under medical treatment for (traumatic) brain injury, recurrent falls, major concurrent injuries, residence abroad or far away (not able to attend follow-up (FU) visits) and contraindication for a 3 Tesla MRI (e.g. pace maker, pregnancy). The CTRL group consisted of 20 healthy age- and sex-matched individuals recruited from the community with normal cognitive functioning. The study was approved by the local ethics Committee. All participants provided written informed consent.

2.1. Procedure for mTBI patients

After providing study information and collecting informed consent, the neurosurgeon on call completed a standardized concussion evaluation form, adapted according to the Acute Concussion Evaluation (ACE) form [30]. Additionally, medical history, current medication, smoking habits and drug use was assessed. As part of standard care of our hospital, an information sheet on mTBI with behavioral advice after mTBI and a prescription for a regular analgesic were handed out prior to discharge.

2.2. Neuropsychological assessments (NPA)

All patients returning to FU were assessed with a battery of validated neuropsychological measures within one week (T1), at three (T2) and twelve months (T3) post-injury. The following tests and questionnaires were included: Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT); subtests alertness, divided attention, covert shift of attention

of the Test of Attentional Performance (TAP); Trail Making Test A and B (TMT); Deux Barrages (DB); visual and verbal retentiveness test (VVM), subtests digits forward, backward span, similarities from the German version of the Wechsler Adult Intelligence Scale (WIE); Five-Point Test (FPT); Regensburg Word Fluency Test (RWT); Stroop Color and Word Test (SCWT); Grooved Pegboard; Green's Medical Symptom Validity Test (MSVT); Adjustment Disorder New Module (ANMD); Beck Anxiety Inventory (BAI); Beck Depression Inventory (BDI); health survey short form (SF 36); stress inventory (SVF).

The patients rated the severity of symptoms on a 7-point Likert scale as part of ImPACT, which was used to compute the post concussion symptom score (PCSS). Raw data, in addition to comparison, were matched to validated data stratified for age and transferred into one single unit (T-scores) and composite indices of attention, memory, executive function, fine motor speed and intellectual capacity. A mean value composite index was calculated as a measure of overall cognitive test performance for each participant at all time points. Additionally, information about the current job, medication and current drug consumption was obtained.

2.3.   MRI data acquisition and analysis

MR-images were acquired at baseline and 12 months after injury in the same 3T Siemens MAGNETOM Verio scanner (Siemens Medical Solutions, Malvern, PA, USA). The MRI protocol consisted of an axial 3D T1-weighted, a fast gradient T2-weighted, a high-resolution 3D gradient-echo SWI and an axial DTI acquisition.

2.4.   Analysis of T1-weighted, fast gradient T2-weighted and SWI sequences

The neuroradiologist recorded the amount and localization of Mb (max 2–10 mm) on SWI. In order to exclude other possible lesions mimicking Mb, the expected Mb should not have a T2-hyperintense rim (edema), should not appear as a calcifications in CT or localize in typical calcification areas (globus pallidus, pineal gland), and should be rather in the typical localizations (border zone gray and white matter, brainstem, corpus callosum). Other findings such as contusion, hematoma, edema or incidental findings were also recorded. Group differences regarding neuropsychological test results and PCSS were calculated between mTBI with MR positive findings and mTBI with MR negative findings. Quantitative correlations between Mb and cognitive performance and symptoms of PCS were calculated.

2.5.   Analysis of DTI data

Semi-automated methods from the FSL toolbox have been used to allow investigating the whole brain without the need of manual segmentation of regions of interest (ROI) [31]. First, Eddy Current and Linear Motion Correction were performed by aligning all the DWI volumes to the image without diffusion-weighting using 12 degrees of freedom. The brain was segmented using the brain extraction tool and the tensor model was fit in every voxel with the DTIFIT program to estimate fractional anisotropy (FA), mean diffusivity (MD),

axial diffusivity (AD) and radial diffusivity (RD) maps [32]. Voxelwise statistical analysis of the DTI derived maps was carried out using tract-based spatial statistics (TBSS) [33]. All subjects' FA maps were non-linearly aligned into a common space (FSL's FMRIB58_FA template) using the registration tool FNIRT [34–36]. Thereafter, the mean FA image was created and thinned to create a mean FA skeleton that represents the centers of all tracts common to the group. Each subjects' aligned FA data were then projected onto this skeleton. A skeleton threshold of 0.2 was used. The resulting data were fed into voxelwise cross-subject statistics. For group comparison, randomized tests on skeletonized DTI images were conducted with threshold-free cluster enhancement (TFCE) procedure and 10,000 permutations (FSL recommended parameters for permutation tests). Statistical results were generated with the family-wise error (FWE) correction for multiple comparisons. The same procedure was applied to MD, AD and RD maps. In addition, a voxel-based morphometry (VBM)-like analysis of the DTI maps was performed (VBM-DTI). Whole brain FA, MD, AD and RD maps were registered to the FMRIB58_FA template, smoothed with a Gaussian kernel of sigma = 3 mm and then fed into the RANDOMISE command of FSL as described above for the TBSS analysis.

Randomize step: Voxelwise general linear model (GLM) was designed with age and sex as covariates. We tested for differences in DTI parameters between T1 and controls, between T3 and controls as well as differences between T3 and T1. The threshold of resulting t-stat FWE corrected maps were set at a significance level alpha = 0.05.

Region by region analysis: FA, MD, AD and RD values in significant voxels were extracted to compute their correlation with clinical data. Furthermore, a slightly modified version of the MNI structural brain atlas was used to identify the brain areas with VBM-DTI differences and to compute mean FA, MD, AD and RD.

2.6.   Statistical analysis

Statistical analysis was conducted using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA). Group comparisons were performed using unpaired, two-tailed Student's t-tests or Mann–Whitney U test. To evaluate the relationship between two categorical variables Pearson's chi-squared test (X^2) and Fisher's exact test were used. Correlations were calculated with Pearson or Spearman correlation. P values <0.05 were considered statistically significant.

3.   Results

The full sample of the present study consisted of 50 participants: 30 mTBI patients (mean age: 35.0, SD: 13.4, range: 18–55 years, 16 males and 14 females) and 20 healthy controls (mean age: 43.2, SD: 14.4, range: 19–62 years, 10 males and 10 females). There were no group differences between mTBI patients and controls regarding sex, age, years of education and handedness. Complete MRI data of 30 patients and 20 patients were available at T1 and T3, respectively. DTI data were available of 29 patients at T1 and of 18 patients at T3. The MR data of one healthy control were excluded due to poor

image quality. A complete NPA could be performed in 27 patients at T1, 24 at T2 and 20 at T3. The NPA data of one patient were excluded since he failed in the MSVT screening for bad effort or malingering. Dropouts were mainly due to motivational reasons of the patients. Time to FU was 0–7 days at T1 (MD: 2.7, SD: 1.8), 81–123 days at T2 (MD: 95.9, SD: 9.2) and 355–406 days at T3 (MD: 372.8, SD: 13.0).

3.1. Injury related MR findings and the presence of Mb

Patients showed a trend of more MR abnormalities at T1 than at T3 considering possible injury related findings (T1 sum of MR findings = 10, T3 sum = 4, Wilcoxon-test = –1. 51, p = 0.132). MR findings of both groups are shown in Table 1. At T1, 16/30 patients showed MR findings possibly associated with the mTBI (Mb, contusion, hematoma, edema). At T3, in 4/20 patients Mb were detected as the MR finding possibly associated to trauma. In two patients Mb were found at T3, but not at T1. One of them reported that he had suffered a second mTBI four months after the initial mTBI. The possible origin of the new Mb in the other patient is unknown. Interestingly, hemosiderin deposits were found in 5 of 19 healthy controls (26.3%) as an incidental finding. The hemosiderin deposits in 2 controls related to calcifications, in 1 to microangiopathy and in 2 it was unknown.

3.2. DTI-analyses

Table 2 shows DTI-parameter analyses of FA, AD, RD and MD between mTBI patients and controls. There were no differences detected at T1 between patients and controls. At T3 FA (TBSS) was increased and RD and MD (TBSS) were decreased in patients. In VBM-DTI findings were similar to TBSS. FA was increased at T3 in patients; AD, RD and MD were decreased in comparison with controls. Fig. 1 shows the significant

differences of DTI parameters between the acute and late phase in mTBI-patients. The TBSS analysis showed greater AD values in the acute phase (T1) compared to the late phase (T3) post-injury in the group of mTBI patients. The VBM-DTI technique revealed consistently significantly higher values in FA, MD and AD in several areas in the acute phase compared to the late phase.

3.3. Correlations between MR findings and NPA data

Symptom values measured with the PCSS were significantly higher in mTBI patients with than without MR findings possibly associated with injury at T1, T2 and T3 (see Table 3). Cognitive testing and psychological questionnaires did not show significant differences between these groups at either FU. Patients with Mb (Mb 1) and without (Mb 0) showed significant group differences in eight neuropsychological test results at T1 (five of them measuring psychomotor speed and speed of information processing), in one test result at T2 and in five test results at T3 (see Table 4). In all but one of the test scores with significant group differences, Mb 1 performed worse than Mb 0. The Mb1 group scored better at the reaction time composite score of the ImPACT. Regarding PCSS, the Mb 1 group showed higher symptom values in total symptom score, fatigue, difficulty concentrating and difficulty remembering at T3 (see Table 4 for details). The amount of Mb at T1 correlated significantly with ten symptom values of the PCSS at T2 and with six symptom values at T3 – all correlations were positive. This means that a higher amount of Mb at T1 was associated with higher symptom severity later on. The amount of Mb also showed a negative correlation with some of the NPA scores at T1, T2 and T3 (see Table 5).

At T1, no significant differences in TBSS or VBM-DTI could be shown between patients and controls. Consequently, no correlations existed between DTI parameters and NPA.

Table 1 – MRI findings of mTBI-patients versus controls.			
Number of patients/controls with:	Frequency (%)		
	T1	T3	Controls
No cerebral MRI findings:	14 (46.7)	14 (80.0)	9 (45.0)
Cerebral MRI findings[b]	16 (53.3)	6 (35.0)	11 (55.0)
Hemosiderin deposit/microbleeds[a]	4 (13.3)	4 (20.0)	5 (25.0)
Contusion[a]	1 (3.3)	0	0
Hematoma[a]	8 (26.7)	0	0
Subdural hematoma[a]	2 (6.7)	0	0
Epidural hematoma[a]	1 (3.3)	0	0
Preseptal/supraorbital hematoma[a]	1 (3.3)	0	0
Subgaleal hematoma[a]	4 (13.3)	0	0
Subarachnoid hematoma[a]	2 (6.7)	0	0
Edema[a]	2 (6.7)	0	0
Foci of gliosis	5 (16.7)	2 (9.1)	6 (30.0)
Calcifications	3 (10.0)	1 (4.5)	3 (15.0)
Moderate cerebral atrophy	1 (3.3)	1 (3.3)	0
Unclear malformations	0	0	2 (10.0)
Total patients	30 (100)	20 (100)	20 (100)

[a] Microbleeds, contusion, hematoma, edema in mTBI patients were associated with the mTBI.
[b] At T1: 1 patient with MRI finding had just one type of MRI finding, all other had more than one type of MRI finding.

Table 2 – Differences in DTI parameters between controls (CTRL) and mTBI patients at T1 and T3.

Technique	Image type	CTRL vs mTBI at T1	CTRL vs mTBI at T3	Regions with sign. group differences	mTBI at T1 vs mTBI at T3	Regions with significant group differences
TBSS	FA	No differences	CTRL<mTBI at T3	Right: white matter	No differences	
	AD	No differences	No differences		mTB at T1> mTBI at T3	Bilaterally: in white matter, parietal and occipital lobe
	RD	No differences	CTRL> mTBI at T3	Bilaterally: in white matter, frontal, parietal, temporal and occipital lobe Right: caudate nucleus, putamen, thalamus	No differences	
	MD	No differences	CTRL> mTBI at T3	Bilaterally: in frontal, parietal, temporal and occipital lobe, caudate nucleus, putamen, white mater	No differences	
VBM-DTI	FA	No differences	CTRL< mTBI at T3	Left: parietal lobe	mTB at T1> mTBI at T3	Bilaterally: in white matter, thalamus Left: caudate nucleus
	AD	No differences	CTRL> mTBI at T3	Bilaterally: in white matter	mTB at T1> mTBI at T3	Bilaterally: white matter, parietal, temporal and occipital lobe, insula Left: frontal lobe, putamen
	RD	No differences	CTRL> mTBI at T3	Bilaterally: in white matter Right: frontal, parietal and temporal lobe	No differences	
	MD	No differences	CTRL> mTBI at T3	Bilaterally: in white matter, Right: frontal, parietal & temporal lobe	mTB at T1> mTBI at T3	Bilaterally: white matter, Left: frontal, parietal and occipital lobe

TBSS, tract-based spatial statistics; VBM-DTI, voxel based morphometry (on DTI data); FA, fractional anisotropy; AD, axial diffusivity; MD, mean diffusivity; RD, radial diffusivity; vs, versus.

3.4.　Mb and development of DTI abnormalities

Patients with Mb at T3 showed significantly higher values in the following DTI parameters of TBSS compared to patients without Mb: MD in left parietal lobe ($t = 2.35$, $p = 0.032$) and in right temporal lobe ($t = 2.32$, $p = 0.034$) and RD in right occipital lobe ($t = 2.53$, $p = 0.022$). The amount of Mb at T3 correlated positively with twelve DTI parameters (MD and RD from several areas) from TBSS, respectively with four DTI parameters (AD, MD and RD in white matter) from VBM-DTI.

4.　Discussion

4.1.　Microbleeds in SWI

Our data show that the amount of Mb measured by SWI in the acute phase correlates positively with cognitive symptoms such as slowing, difficulty in memory and concentration after mTBI. Our data support previous findings of associations of MR abnormalities and acute symptoms as well as correlations between Mb and a lower GOS at 1 year and development of PCS, suggesting important prognostic value for persistent symp-

toms [13,37–39]. However, we could not find a significant correlation between the amounts of Mb between patients with PCS compared to patients without PCS. Due to the low number of patients with Mb no functional anatomical correlations were found in the current study. Presence and quantity of Mb were previously found to be closely related with lower scores on the GCS on the day of trauma and on the GOS one year post-injury [37].

Differences in cognitive performance in the acute phase in patients with Mb have been most commonly found in cognitive tests measuring psychomotor speed and speed of information processing showing opposite results [34]. In the current study, there is no logical explanation for the better performance of patients with Mb in the reaction time composite score of ImPACT in comparison to patients without Mb.

Our age-matched controls showed unexpected high rates of Mb, which puts the entity "traumatic microbleed" into another perspective and could have led to a Type II error in the mTBI population. Akoudad et al. showed in a large population based study in >60 years old subjects the predictive power of microbleeds in ischemic brain lesions [40]. Next to that, Mb are predictive of both intracerebral hemorrhage as
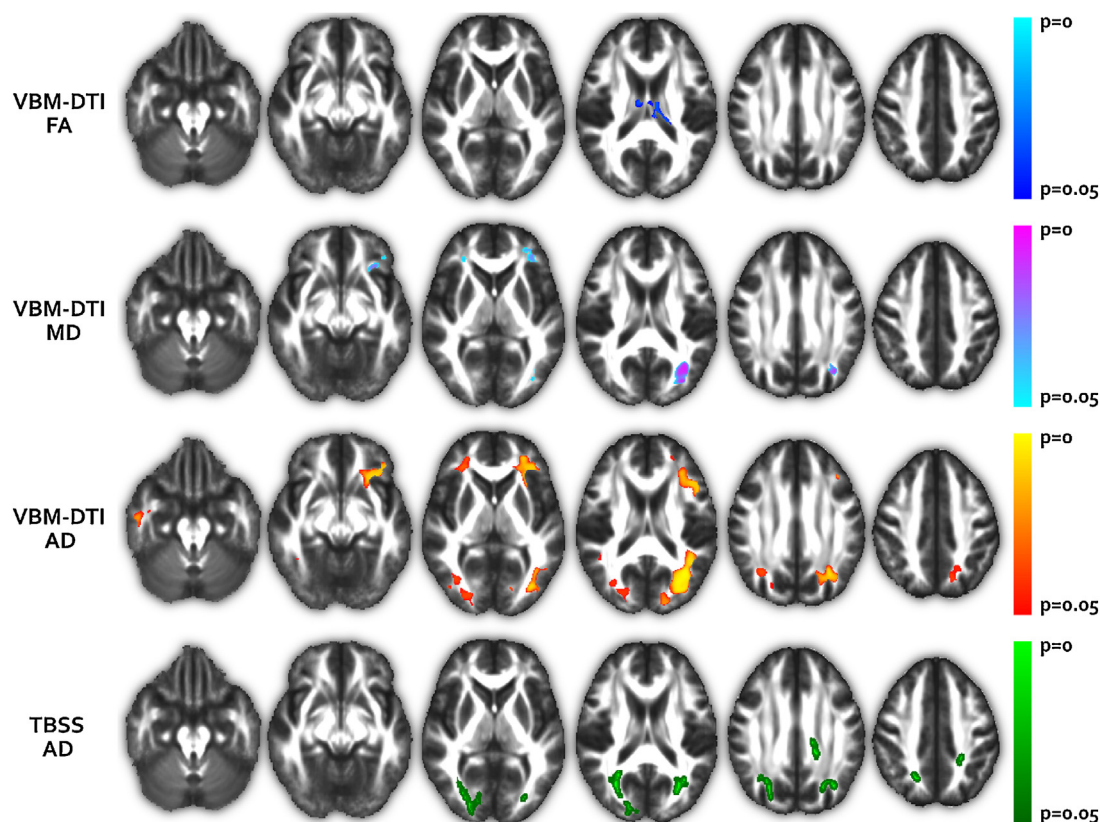
Fig. 1 – Differences in DTI parameters in the acute phase in mTBI-patients. The colored regions are the regions that showed a difference in comparison to the chronic phase. Blue, turquoise and yellow areas in 1st–3rd row show where VBM-DTI revealed higher values of FA, MD and AD in the acute phase, respectively. Green clusters in the bottom row show areas where TBSS revealed higher values of AD in the acute phase. VBM, voxel-based morphometry, and TBSS, tract-based spacial statistics, findings in the acute phase versus late phase in mTBI patients.

Table 3 – Neuropsychological differences between patients with versus patients without MRI findings at T1 in PCSS at T1–T3.				
	mTBI with MRI findings M (SD)	mTBI without MRI findings M (SD)	U	p
T1				
Feeling slowed down	3.00 (1.51)	1.35 (1.94)	32.00	0.037
Difficulty remembering	3.00 (1.60)	1.00 (1.80)	27.00	0.016
T2				
Difficulty concentrating	3.00 (1.00)	0.13 (0.50)	0.50	0.002
T3				
Difficulty concentrating	1.29 (1.70)	0.17 (0.58)	0.50	0.002

well as cerebral small vessel disease [41]. The development of Mb after mTBI could thereby be influenced by other factors than the actual mechanical injury itself, which should be taken into consideration when conducting SWI-sequences after an mTBI.

4.2. Structural integrity measured by DTI

Diffusion Tensor Imaging (DTI) is a newer technique that is under investigation to detect DAI than conventional MR imaging [42]. It allows detection of changes in the white matter tracts and brain structural connectivity [19]. In the present study, correlations between higher numbers of Mb and higher values in the DTI parameters MD, AD and RD were found. Whether structural damage is caused by the Mb due to weaker constraining of water molecule movements and more freely moving water is not known and needs further investigation. Mb were not excluded from the DTI analyses, as they are not detectable in T2 or DTI. However, one would expect lower values of MD, AD and RD, which were not found. In the acute phase (1 week), no significant differences in DTI patterns of mTBI patients compared to healthy controls were found. A possible explanation is that DTI in the acute phase is not sensitive enough to detect true differences.

Table 4 – Neuropsychological differences between mTBI-patients with and without microbleeds.				
Tested cognitive skill (units) – test name	Mb+ mean (SD)	Mb− mean (SD)	U	p
T1				
Verbal fluency lexical (words per min) – RWT	6.33 (0.58)	14.55 (5.04)	5.00	0.014
Verbal fluency categorical (words per min) – RWT	17.00 (2.65)	23.73 (6.26)	7.00	0.027
Design fluency (items per 3 min) – Design Fluency	22.67 (3.51)	33.13 (7.99)	7.00	0.024
Fine motor speed dominant hand (sec. till completion) – Grooved Pegboard	93.67 (22.50)	63.05 (7.91)	4.50	0.011
Fine motor speed non-dominant hand (sec. till completion) – Grooved Pegboard	90.67 (16.65)	72.19 (10.86)	7.50	0.031
Delayed verbal recall (number of recalled items) – VVM	3.67 (2.89)	9.87 (5.51)	9.00	0.041
BDI-II severity coding (1 = minimal, 2 = mild, 3 = median, 4 = sever depressive symptom) – BDI-II	1.33 (0.578)	0.30 (0.56)	7.50	0.024
Reaction time composite score (T-score) – ImPACT	51.33 (5.13)	43.09 (6.26)	9.50	0.046
T2				
Divided attention visual cue (mean reaction time in sec.) – TAP	827.00 (43.14)	724.85 (74.46)	7.00	0.035
T3				
Extrinsic alertness (mean reaction time in sec.) – TAP	241.50 (0.71)	219.53 (15.45)	2.00	0.047
Speed of processing (sec. till completion) – TMT A	25.50 (0.71)	16.71 (4.44)	2.00	0.047
Immediate verbal recall (number of recalled items) – VVM	9.50 (0.71)	15.53 (3.81)	2.00	0.047
Fine motor speed dominant hand (sec. till completion) – Grooved Pegboard	74.50 (9.19)	55.76 (4.80)	0.00	0.012
Composite score attention (T-score) – mean of all tests measuring attention	46.50 (1.56)	52.14 (2.38)	0.00	0.012
PCSS				
Total Symptom Score	12.29 (12.87)	5.00 (4.24)	1.50	0.023
Fatigue	1.86 (1.95)	0.58 (1.17)	1.50	0.023
Difficulty concentrating	1.29 (0.70)	0.17 (0.58)	0.00	0.012
Difficulty remembering	1.71 (2.22)	0.00 (0.00)	0.00	0.012

Mb+, with microbleeds; Mb−, without microbleeds. Lower values indicate better performance in the following tests: Grooved Pegboard, BDI-II, TAP, TMT A, PCSS. Higher values indicate better performance in the following tests/composite scores: RWT, Design Fluency, VVM, ImPACT, overall performance for the domain attention. In PCCS a higher score reflects a higher symptom severity. Per symptom 6 was the highest selectable value, 0 means the patient does not experience a symptom.

Interestingly, after 1 year higher values in FA and lower values in MD, RD (TBSS and VMB-DTI) and AD (VBM-TBI only) compared to healthy controls and higher values in FA, MD and AD (VMB-DTI) and AD (TBSS) compared to the acute phase were found in widespread regions, possibly reflecting axonal atrophy in the late phase. In the TBSS analysis, no differences were found in AD for the late phase after mTBI compared to controls, which is in accordance with the finding of Messè et al. [22]. Higher FA in mTBI has been related to an inflammatory response such as axonal swelling or cytotoxic edema [21,43,44]. Our pattern of higher values in FA in the acute phase compared to the late phase in the same patients with mTBI resembles the finding of Veeramuthu et al., that also found higher values in FA in their mTBI group in the acute phase (within 24 h) compared to a different chronic phase (after six months) [24]. Our pattern of significantly higher MD and AD in the acute phase compared to the late phase (after 12 months) in the same group of mTBI patients is a new finding, since other studies comparing mTBI at a similar time point did not find significant group differences in MD or AD [24,27,28]. Chronic pathophysiological pathways are triggered leading to DTI changes at a later stage as shown in the current study and by Yuh et al. [45]. As the disconnection is not present, or at least not detectable in the acute phase compared to controls, it is probable that a chronic process leads to destruction of neuronal pathways and gliosis as seen in all peripheral and central nerve injuries. The theory that Mb release iron increasingly into the tissue and thereby leading to further destruction, possibly leading to triggering inflammatory pathways as well is still to be proven [46,47]. These findings call for further investigations comparing diffusion parameters between mTBI patients in the acute and late phase in larger populations.

4.3. Limitations

Our findings are limited by the small sample size. It was primarily due to lack of patient motivation to participate in spite of active telephone follow-ups, reimbursement of travel costs and strict inclusion criteria. Due to the lack of NPA of the healthy control group we were not able to analyze the neuropsychological effects of Mb as a degenerative disorder, e.g. small vessel disease, in absence of mTBI.

Despite all efforts, numerous factors influence the validity in the comparison of DTI data; e.g. selection of mTBI patients (i.e. especially with or without CT findings), type of scanner, scan parameters, time of scan post-injury and type of method of analysis (e.g. TBSS or VBM-DTI). It has also been shown that more diffusion directions lead to more accurate estimation of the diffusion tensor, hence the choice to use 64 directions in this study was valid [48]. From a technical point of view more $b = 0$ images should have been acquired in order to improve bias of fiber orientation.

Table 5 – Correlations between number of Mb at T1 and performance on neuropsychological tests and PCSS symptom severity.

Tested cognitive skill or symptom – test name	R	p
T1		
Word fluency lexical – RWT	−0.482	0.015
Word fluency semantic – RWT	−0.398	0.049
BDI-II severity coding – BDI-II	0.523	0.006
T2		
BDI-II severity coding – BDI-II	0.459	0.032
PCSS		
Total Symptom Score	0.477	0.025
Nausea	0.500	0.018
Dizziness	0.535	0.010
Sensitivity to noise	0.835	0.000
Sleeping more than usual	0.425	0.049
Feeling more emotional	0.474	0.026
Sadness	0.474	0.026
Nervousness	0.724	0.000
Difficulty concentrating	0.513	0.015
Difficulty remembering	0.560	0.007
T3		
Speed of processing – TMT A	−0.517	0.028
Fine motor speed dominant hand – Grooved Pegboard	−0.584	0.011
Composite score attention – overall performance for the domain attention	−0.547	0.019
BDI-II severity coding – BDI-II	0.728	0.001
PCSS		
Total Symptom Score – PCSS	0.504	0.033
Nausea	0.500	0.035
Visual problems	0.728	0.001
Fatigue	0.570	0.013
Difficulty concentrating	0.841	0.000
Difficulty remembering	0.835	0.000

5. Conclusions

Microbleeds in the acute phase of mTBI were associated with later cognitive symptoms and the development of PCS, however the Mb can also be caused by ischemic disease as described previously. Structural integrity measured by DTI is more affected by mTBI in the late phase than in the acute phase, implying late structural reorganizing processes. SWI seems to be more valuable in the acute phase than DTI. Hence we recommend performing an MRI including SWI sequences if an mTBI patients' recovery develops unfavorably within the first few weeks after mTBI. The goal thereafter is depending on SWI results to recommend neuropsychological testing and tailored treatment. Further studies are needed to assess a clinical benefit of DTI in mTBI patients in the chronic phase.

Conflict of interest

None declared.

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