Table 2
Characteristics of estimated sources determined by equivalent current dipole (ECD) and quantitative modification of standardised low-resolution brain electromagnetic tomography (sLORETA-qm).

	Distance between the two sources* [median (range)] (mm)	Intensity range (nAm)
ECD	3.53 (1.28–4.98)	72.35–767.62
sLORETA-qm		62.89–716.54

* (a) in Fig. 1.

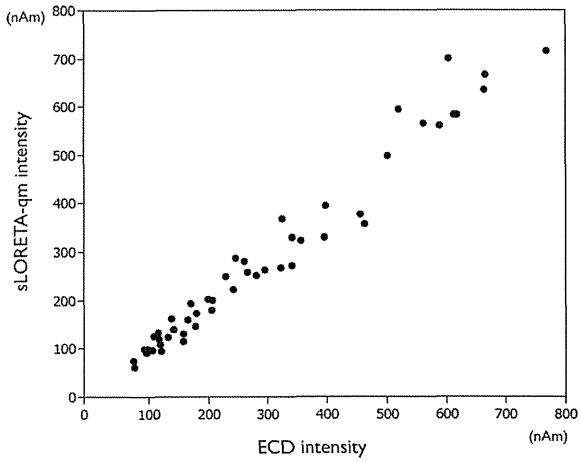

Fig. 3. The correlation between intensities from equivalent current dipole (ECD) and quantitative modification of standardised low-resolution brain electromagnetic tomography (sLORETA-qm) is depicted as a scatter plot. A close correlation is found between the two values (Spearman's correlation coefficient, Rho = 0.9803, $p < 0.001$).

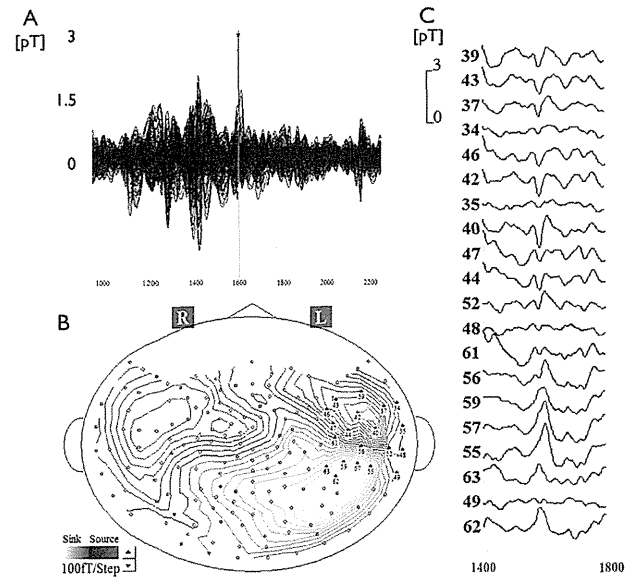

Fig. 4. (A) An overlaid waveform of all 160 sensors between 1000 ms and 2200 ms is demonstrated. High amplitude magnetic flux was recorded around 1597 ms. (B) Isofield contour map at the time point of 1597 ms is depicted. The maximum extremum (red contour) and minimum extremum (green contour) are recognised on the left temporal area. Blue dots indicate the 20 selected sensors used for analysis of the equivalent current dipole (ECD). (C)Waveforms of 20 selected sensors are shown.

centre was 55.94 mm. Estimated sources from the two analytical methods were superimposed on the patient's brain (Fig. 6). In this figure, the estimated source and orientation by ECD are depicted with a blue square and tail, respectively. The estimated peak source by sLORETA-qm is depicted as pink and white ellipse. The distance between the two sources is 4.33 mm.

4. Discussion

The present study evaluated the relationship between the sLORETA-qm and ECD concerning source location and quantity of the interictal epileptic discharge. In the analysed range of intensities (approximately 70–700 nAm), ECD dipole moments and sLORETA-qm intensities were closely correlated. There was no significant tendency concerning the distance from the centre of the spherical model between the two methods. To our knowledge, there have been no previous reports that have compared sLORETA-qm and ECD point source localisation and quantification during analysis of the interictal spike discharge in MEG.

ECD is a conventional and widely used approach, especially for studying the interictal epileptic spike discharge (Assaf et al., 2004; Iwasaki et al., 2002; Sakamoto et al., 2003). However, there are several problems concerning application of the ECD model to interictal epileptic spike discharge. The main problem is derived from the fact that all sensors cannot be taken into account when estimating the ECD source. In addition, the number of sources must be assumed by the isofield contour map before fitting the dipole model. Sensor location and the number of sensors selected depend on the examiner's experience and preference. Subjective and objective assessments of the irritative zone from the patient's ictal symptom may also affect sensor selection. Consequently, source location and quantity can be arbitrarily changed by each examiner, especially in the case of multiple sources. Another problem is that the sources are estimated as a maximum intensity spot, not a distributed area as in ECD. Considering that epileptic activities are often associated with multiple sources, and they are usually distributed in some areas of the brain, the use of spatial filtering in analysis of epileptic activities may be appropriate.

One of many kinds of spatial filtering techniques, sLORETA was adopted in the present report. sLORETA is one of the non-adaptive beamformer methods. Although non-adaptive beamformer methods generally have low resolution compared with adaptive beamformer methods (Sekihara and Nagarajan, 2008), there are two advantages with this type of technique for analysing the interictal spike. First, non-adaptive beamformer methods can reconstruct the source image from a single time point, but beamformer methods invariably need time course information for weight calculation in each voxel. Considering that the epileptic spike is instant electrical activity in the brain, non-adaptive beamformer methods may be more appropriate spatial filtering for interictal spike activity. Second, sLORETA can provide a quantitative source power with the unit of current intensity (nAm); otherwise, beamformer methods only offer a relative statistical value. This is advantageous when comparing the source power with ECD.

In spatial filtering techniques, including sLORETA-qm, signals from deeply located voxels are usually enhanced compared to signals in superficially located voxels. Therefore, in order to assess the tendency of source locations, the distance from the spherical centre was compared between these two methods. As a result, there were no significant differences between the two distances (paired sample *t*-test, $p = 0.8761$). To prevent false negative (beta) errors, a *post hoc* power analysis (using alpha = 5%) was performed on the data in order to calculate the required sample size for detecting a relevant difference. A relevant difference was defined as more than 2.5 mm (half of the voxel size) distance between

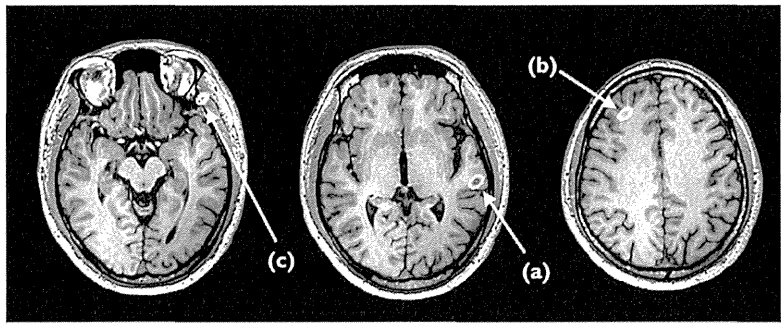

Fig. 5. A total of three sources estimated by quantitative modification of standardised low-resolution brain electromagnetic tomography (sLORETA-qm) are shown in pink and white ellipses. The source intensities are 164.05, 66.19 and 39.80 nAm for (a), (b) and (c), respectively. The peak voxel is located in the left temporal lobe (a), and the distance from the spherical centre is 55.94 mm.

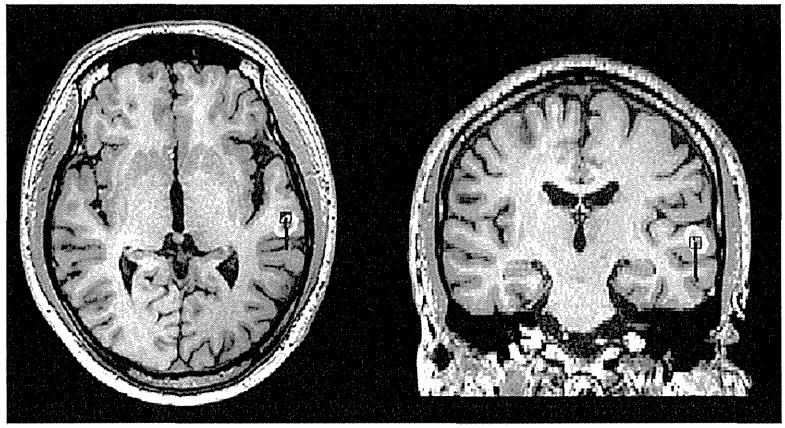

Fig. 6. Sources estimated by two analytical methods superimposed on the patient's brain. Blue square and tail indicate the estimated source and orientation by ECD, respectively. Pink and white ellipses indicate the estimated source by standardised low-resolution brain electromagnetic tomography (sLORETA-qm). The distance between the two sources is 4.33 mm.

the two methods. This analysis showed that the present study had sufficient power to detect a relevant difference at the 99.9% level. Consequently, the sample size ($n = 50$) in the present study was considered to be large enough for detection of a relevant difference between groups.

In a previous report, SEF intensities determined by ECD and sLORETA-qm were significantly correlated in the range of approximately 10–70 nAm (Terakawa et al., 2008). The analysed range of the source intensities in the present study was approximately 70–700 nAm, and close correlation between the intensities determined by the two methods was demonstrated with the Spearman's correlation coefficient. Thus, intensities obtained by sLORETA-qm may be reliable and quantitative over a wide range of intensities of paroxysmal interictal epileptic discharge.

A disadvantage of sLORETA-qm is that sources without clinical significance are often estimated. In the representative case in the present study, a total of three sources were estimated at the selected time point. The peak source amongst the estimated sources by sLORETA-qm corresponded to the source by ECD, and it could be distinguished as a clinically significant source. However, especially in the more complex isofield contour patterns, such as spatially close or synchronised multiple sources, it may be difficult to distinguish clinically significant sources among the estimated sources. This is one of the limitations of the spatial filtering technique. If the number of estimated sources is limited to one in the sLORETA-qm analysing process, only one source will be obtained and that source will be corresponded with the source by ECD. However, limiting the number of sources in analysis is somewhat arbitrary. Therefore, the number of estimated sources was not limited in the present study.

sLORETA-qm has a methodological limitation. The analytical results of sLORETA-qm depend on voxel size. The estimated localisation of the MEG sources can be restricted by its lattice, which can result in some localisation error. In the present study, the voxel diameter was 5 mm and the possible maximum localisation error derived from the voxel setting was 4.33 mm (the distance between the centre and apex of a voxel). This was because concordant sources were defined as a distance between the two sources of less than 5 mm, and only the concordant sources were adopted for the analysis.

In the present study, in order to verify the performance of sLORETA-qm, time points with typical maximum extremum and minimum extremum depicted on the isofield contour map were selected for analysis. For future studies, more complex isofield contour patterns should be analysed with sLORETA-qm. Although multiple sources are often difficult to analyse using only ECD, added use of sLORETA-qm may be helpful for evaluation of these complex epileptic spike discharges.

5. Conclusions

sLORETA-qm showed a close correlation with ECD in point source location and quantity for analysis of the interictal epileptic

spike, and is a reliable quantifiable method without arbitrariness for analysis of the interictal epileptic spike.

Acknowledgement

None of the authors has any conflicts of interest to disclose.

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ORIGINAL COMMUNICATION

Fractional anisotropy in the centrum semiovale as a quantitative indicator of cerebral white matter damage in the subacute phase in patients with carbon monoxide poisoning: correlation with the concentration of myelin basic protein in cerebrospinal fluid

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Abstract Carbon monoxide (CO) poisoning leads to demyelination of cerebral white matter (CWM) fibers, causing chronic neuropsychiatric symptoms. To clarify whether fractional anisotropy (FA) from diffusion tensor imaging in the centrum semiovale can depict demyelination in the CWM during the subacute phase after CO inhalation, we examined correlations between FA in the centrum semiovale and myelin basic protein (MBP) in cerebrospinal fluid. Subjects comprised 26 adult CO-poisoned patients ≤60 years old. MBP concentration was examined for all patients at 2 weeks after CO inhalation. The mean FA of the centrum semiovale bilaterally at 2 weeks was also examined for all patients and 21 age-matched healthy volunteers as controls. After these examinations, the presence of chronic symptoms was checked at 6 weeks after CO poisoning. Seven patients displayed chronic symptoms, of whom six showed abnormal MBP concentrations. The remaining 19 patients presented no chronic symptoms and no abnormal MBP concentrations, with MBP concentrations undetectable in 16 patients. The MBP concentration differed significantly between patients with and without chronic symptoms. The mean FA was significantly lower in patients displaying chronic symptoms than in either patients without chronic symptoms or controls. After excluding the 16 patients with undetectable MBP concentrations, a significant correlation was identified between MBP concentration and FA in ten patients. The present results suggest that FA in the centrum semiovale offers a quantitative indicator of the extent of demyelination in damaged CWM during the subacute phase in CO-poisoned patients.

Keywords Carbon monoxide poisoning · Cerebral white matter fiber · Demyelination · Diffusion tensor imaging · Fractional anisotropy · Myelin basic protein

Abbreviation

CNS	Central nervous system
CSF	Cerebrospinal fluid
CO	Carbon monoxide
COHb	Carboxyhemoglobin
DNS	Delayed neuropsychiatric sequelae
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
ADC	Apparent diffusion coefficient
GCS	Glasgow coma scale
MBP	Myelin basic protein

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MRI Magnetic resonance imaging
ROI Region of interest
T2WI T2-weighted magnetic resonance imaging

Introduction

Approximately 30% of patients surviving acute carbon monoxide (CO) poisoning display various chronic neuropsychiatric symptoms [31, 32]. Of these, approximately two-thirds demonstrate persistent neurological symptoms from the acute phase to the chronic phase. The remaining one-third show delayed neuropsychiatric sequelae (DNS), which are recurrent neuropsychiatric symptoms occurring after an interval of apparent normality ("lucid interval;" mean duration 22 days) following apparent recovery from acute symptoms [6, 33]. Animal experiments and some clinical studies have led to the hypothesis that damage after CO poisoning results from complicated mechanisms due to CO-mediated toxicity: mitochondrial oxidative stress in the central nervous system (CNS) following CO-induced tissue hypoxia [35]; perivascular oxidative stress mediated by intravascular neutrophil activation [26]; and alteration of myelin basic protein (MBP), a major myelin component in the CNS, due to lipid peroxygenation leading to auto-immunological demyelination of CNS [24, 25]. Auto-immunological demyelination induces further inflammation in the cerebral white matter (CWM) [31]. Gray matter structures, such as the cerebral cortex, basal ganglia and hippocampus, must be damaged by severe hypoxia, since these structures display higher cellular activity and higher oxygen requirements than white matter structures and are more vulnerable to oxygen deprivation [29]. However, damage in the CWM is seen in patients both with and without damage to gray matter structures, and the severity of CWM damage appears to correlate with prognosis in CO-poisoned patients [15, 34].

Assessment of CWM damage caused by CO poisoning in the acute or subacute phase contributes to predictions of progress to DNS and prognosis of chronic symptoms, and appropriate triage of patients with CO poisoning for observation and treatment. Additional quantitative and objective examinations are desirable for assessment of CWM damage after CO poisoning. However, no universally accepted severity scale in routine examinations, such as level of consciousness or carboxyhemoglobin concentration, is available for assessing CWM damage caused by CO poisoning. This is because clinical features are largely affected by the degree of cellular hypoxia resulting from binding of CO to myoglobin rather than hemoglobin and may be markedly affected by various conditions before admission, such as the duration before hospitalization and the care provided before hospitalization [7, 12, 20]. As one of mechanisms for damage in CWM is auto-immunological demyelination, measuring the MBP concentration in the cerebrospinal fluid (CSF) has recently been proposed as an indicator for the extent of CWM damage after CO poisoning [11, 14]. However, detection of MBP using a lumbar tap is a highly invasive procedure and only indicates white-matter damage somewhere within the entire CNS. A less-invasive, objective and quantitative examination that could be used in place of measuring MBP is therefore desired. Diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) sequence, is potentially more sensitive for detecting demyelination in CWM. Among various quantitative parameters such as apparent diffusion coefficient (ADC) and eigenvalues derived from DTI, fractional anisotropy (FA) has been recognized as the most useful for evaluating the integrity of CWM fibers [2]. Indeed, FA is frequently used for evaluating the extent of damaged CWM fibers in patients with demyelinating diseases such as multiple sclerosis [1, 27]. CO poisoning causes damage in various regions of the CWM, but the centrum semiovale has been considered a region more responsible for chronic neuropsychiatric symptoms after CO poisoning than other regions [4, 10, 19, 22]. Herein, we measured FA from DTI at the centrum semiovale in CO-poisoned patients, and evaluated the correlation between the FA and concentration of MBP in the CSF. This study aimed to clarify whether FA in the centrum semiovale offers a quantitative indicator of the extent of demyelination in damaged CWM during the subacute phase in CO-poisoned patients.

Methods

Patients

All study protocols were approved by the Ethics Committee of Iwate Medical University, Morioka, Japan. Patients recruited to this study were admitted to Iwate Medical University Hospital between April 2008 and February 2011. Entry criteria for this study were: age $\geq$20 but $\leq$60 years in patients who had suffered from CO poisoning caused by a fire or charcoal burning; performance of DTI and measurement of MBP concentration according to the protocol in this study; no past history of brain disorders, including surgical operation, irradiation, stroke, infection or demyelinating disease; and provision of written informed consent to participate. Diagnosis was based on present history of exposure to CO and presence of acute neurological symptoms such as impairment of consciousness and headache on admission. After excluding patients

who did not meet the entry criteria, 26 patients were enrolled. Mean duration from the scene of CO exposure to arrival at our institute was 5.0 h (range 0.3–81 h). All patients were treated with hyperbaric oxygenation therapy (HBO_2) (60 min of 100% oxygen inhalation via mask at 2.8 atmospheres absolute) started within 24 h of admission. HBO_2 was continued with a single daily session for a week excluding the weekend. HBO_2 was further continued for 4–8 weeks in cases with persistent symptoms. If DNS occurred, HBO_2 was restarted and continued until 2 months after CO exposure. HBO_2 was discontinued upon patient request or when symptoms were sufficiently improved. Duration of HBO_2 administration for all patients ranged from 1 to 60 sessions (mean 12 sessions). The day of CO inhalation was defined as day 1 in this study.

Measurement of MBP concentration in CSF

MBP concentration in the CSF was examined using a lumbar tap at 2 weeks after CO poisoning (between day 12 and day 16) for all patients. Obtained CSF was frozen at −20°C within 1 h after lumbar tap, then the frozen CSF was transported on dry ice to an outside laboratory (SRL, Tokyo, Japan). MBP in the CSF was assayed and measured using a MBP ELISA kit (Cosmic Corp., Tokyo, Japan) immediately after arrival at the laboratory. If the assay was delayed for a long time, frozen CSF was stored at −80°C. An abnormal MBP concentration was defined as ≥102 pg/ml. When the level of MBP was below the limit of detection, the result from the laboratory was reported as MBP ≤40 pg/ml.

DTI

For all patients, DTI was also performed at 2 weeks (between day 12 and day 16) using a 3.0-T whole-body scanner (GE Yokogawa Medical Systems, Tokyo, Japan) and 8-channel coil. Measurements of FA and ADC were performed using data from DTI (repetition time, 10,000 ms; echo time, 62 ms; matrix 128 × 128; field of view, 240 × 240 mm; 4 mm thickness with 1.5 mm gap; 6 motion-probing gradient directions; b value, 1,000 s/mm^2). The region of interest (ROI) was manually placed in the bilateral centrums semiovale in the CWM on non-diffusion-weighted images (Fig. 1). FA and ADC were measured bilaterally at the centrum semiovale, using free MRIcro software (http://www.cabiatl.com/mricro/). The FA and ADC for each subject were determined as the mean of values measured twice by the same investigator (S.F.), who was blinded to clinical data. The second measurement was performed 1 week after the first test, using a different randomized order of measurements from the first test.

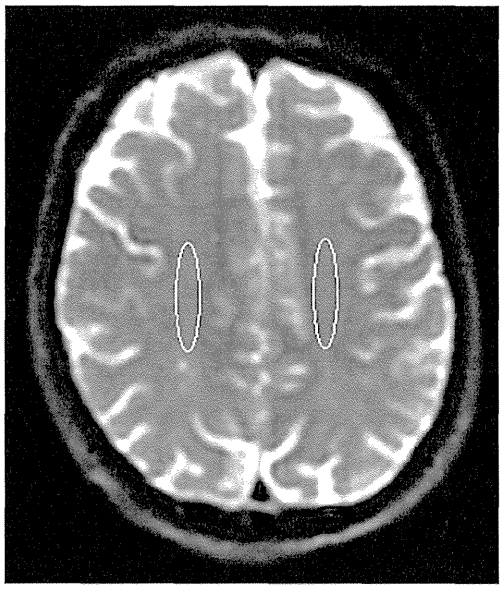

Fig. 1 Measurements of FA and ADC value at the centrum semiovale in a patient (case 7 in group S). Regions of interest (ROIs) were placed bilaterally on the centrum semiovale in non-diffusion-weighted image

Finally, the mean FA and mean ADC values for the right and left centrum semiovale were calculated and defined as absolute values for each subject. The same procedures described above were performed for 21 age-matched healthy volunteers as controls (18 men, 3 women; mean age 41 ± 10 years, range 22–56 years).

Observation of symptoms

Neurological symptoms were continuously observed for 6 weeks after admission using routine neurological examinations. Patients were assigned to one of two groups according to clinical behavior at 6 weeks (day 40–44) after CO poisoning: group S, patients displaying neuropsychiatric symptoms; group A, patients showing asymptomatic status. Group S included both patients with symptoms persisting for 6 weeks and patients with DNS. DNS was defined as recurrent symptoms after apparent improvement of acute symptoms followed by a lucid interval. General intellectual function was also estimated using the mini-mental state examination (MMSE) [9] at 6 weeks after CO exposure. We defined the normal range, borderline range and dementia according to MMSE scores as ≥27, ≤26 but ≥22, and ≤21, respectively. When scores were considered borderline, patients with educational background ≥9 years and evidence of obvious personality change according to interviews with family members were diagnosed with dementia.

Statistical analyses

We statistically compared differences in mean age among group S, group A and controls using the Mann-Whitney U test. The incidence of abnormal ($\geq$102 pg/ml) MBP concentration was compared between the two patient groups (group S and group A) using the χ^2 for independence test. Mean FA and mean ADC values among two patients groups and controls were compared using the Mann-Whitney U test. Intra-operator reliability for all absolute FA and ADC values was evaluated according to classification of the intra-class correlation coefficient (ICC) [21]. For ICC(1,1) and ICC(1,k) as intra-operator reliability, agreement of all absolute values between the first and second tests was analyzed for right and left lesions using one-factor analysis of variance. After excluding patients showing undetectable concentrations of MBP ($\leq$40 pg/ml), the correlation between MBP and mean FA value was estimated using Spearman's correlation coefficient by rank test. Statistical significance was established at the $p < 0.05$ level in all analyses.

Results

A total of 51 patients were admitted to our institute for treatment of CO poisoning between April 2008 and February 2011. After excluding 25 patients who did not meet the entry criteria for this study, a total of 26 patients (24 men, 2 women; mean age 40.1 $\pm$ 11.4 years) were enrolled. All patient data are summarized in Table 1. In 19 (73%) of 26 patients, acute symptoms resolved completely within 4 days after admission, and no neuropsychiatric symptoms were present at 6 weeks from CO-inhalation (group A). The remaining seven patients (27%) displayed chronic neuropsychiatric symptoms at 6 weeks (group S), including four patients with continuous persistence of symptoms for 6 weeks and three patients exhibiting DNS

Table 1 Summary of all patients

Case	Group	Age	Etiology	COHb (%)	GCS	MBP (pg/ml)	Mean FA	Mean ADC	Main symptom at 6 weeks	MMSE score
1	S	29	Suicide	24.8	11	252	0.345	0.622	Dementia (persistent)	23
2	S	57	Suicide	25.1	10	176	0.344	0.548	Parkinsonism (persistent)	27
3	S	38	Suicide	1.5	6	468	0.239	0.494	Apallic syndrome (persistent)	NS
4	S	55	Suicide	39.7	3	376	0.346	0.548	Dementia (persistent)	16
5	S	56	Suicide	13.5	11	130	0.338	0.584	Akinetic mutism (DNS)	NS
6	S	29	Suicide	3.6	14	99	0.353	0.498	Parkinsonism (DNS)	28
7	S	48	Suicide	28.6	6	110	0.317	0.565	Dementia (DNS)	23
1	A	22	Suicide	20.5	15	52.8	0.488	0.492	None	29
2	A	31	Suicide	47.3	13	40.6	0.354	0.494	None	30
3	A	22	Suicide	9.3	12	63.6	0.447	0.555	None	30
4	A	47	Heating	33.3	14	$\leq$40	0.441	0.496	None	30
5	A	44	Heating	13.7	15	$\leq$40	0.388	0.528	None	30
6	A	26	Suicide	1.9	15	$\leq$40	0.395	0.504	None	30
7	A	47	Heating	22.6	14	$\leq$40	0.393	0.551	None	29
8	A	28	Suicide	19.2	15	$\leq$40	0.440	0.521	None	30
9	A	41	Suicide	2.7	11	$\leq$40	0.381	0.487	None	30
10	A	55	Heating	14.0	13	$\leq$40	0.366	0.504	None	30
11	A	35	Suicide	25.3	8	$\leq$40	0.425	0.497	None	30
12	A	56	Suicide	12.2	15	$\leq$40	0.395	0.501	None	30
13	A	36	Suicide	44.1	12	$\leq$40	0.398	0.513	None	30
14	A	34	Suicide	31.0	12	$\leq$40	0.394	0.530	None	30
15	A	57	Heating	40.1	13	$\leq$40	0.400	0.541	None	30
16	A	32	Suicide	19.3	10	$\leq$40	0.358	0.509	None	30
17	A	34	Suicide	38.6	5	$\leq$40	0.406	0.535	None	30
18	A	36	Suicide	23.5	10	$\leq$40	0.358	0.520	None	30
19	A	48	Suicide	44.0	6	$\leq$40	0.352	0.539	None	30

COHb and GCS indicate results of the initial examination

COHb carboxyhemoglobin, *GCS* Glasgow coma scale, *NS* no study performed because of unconsciousness

after apparent improvement of acute symptoms followed by a lucid interval. DNS in three patients occurred after DTI and measurement of MBP on day 21 in case 5, day 19 in case 6 and day 18 in case 7. Mean age was 45 ± 12 years in group S, 38 ± 11 years in group A and 41 ± 10 years in controls. No significant differences in age were found between groups S and A ($p = 0.24$), between group S and controls ($p = 0.51$), or between group A and controls ($p = 0.40$).

In the seven patients in sroup S, six showed abnormal MBP concentrations (≥102 pg/ml), and one patient showed a level of 99 pg/ml. None of the 19 patients in group A showed abnormal concentrations of MBP, with 16 patients showing undetectable concentrations of MBP (≤40 pg/ml). The incidence of an abnormal MBP levels was statistically different between groups P and A ($p < 0.001$). MBP concentrations for the four patients with persistent symptoms in group S, for the three patients with DNS in group S and for the three patients in group A were more than ≥150 pg/ml, around 100 pg/ml and around 50 pg/ml, respectively (Table 1).

Table 2 shows ranges and means of FA and ADC for each group. The range of FA for group S slightly overlapped that for group A, but differed markedly from that for controls. Ranges of FA for group A and controls were similar. The mean FA for group S was significantly lower than those for group A ($p < 0.001$) and controls ($p < 0.001$), whereas no significant difference was found between group A and controls ($p = 0.57$) (Fig. 2a). In Fig. 2a, individual mean FA values of the three patients with DNS were not obviously different from those of the four patients with persistent symptoms in group S. Group S patients were clearly differentiated from group A patients at a cutoff of 0.353 (100% sensitivity, 94.7% specificity) and from controls at a cutoff of 0.360 (100% sensitivity, 100% specificity). On the other hand, the range of ADC in each group was similar, and the mean ADC did not differ significantly among any of the three groups (Fig. 2b). Intra-operator reliability for absolute FA was classified as "almost perfect" for the centrum semiovale bilaterally; ICC(1,1) and ICC(1,*k*) were 0.88 and 0.93 for the right side, and 0.95 and 0.98 for the left side, respectively. Intra-operator reliability for absolute ADC was also classified as "almost perfect" for bilateral centrum semiovale; ICC(1,1) and ICC(1,*k*) were 0.98 and 0.99 for the right side, and 0.91 and 0.95 for the left side, respectively.

After excluding 16 patients showing undetectable levels (≤40 pg/ml), the ten remaining patients (all patients in groups S and 3 patients in group A) showed a strong correlation between the mean FA and MBP ($r = -0.79$, $p = 0.02$) (Fig. 3).

Discussion

Ide et al. [11] have documented that MBP concentration in patients with DNS showed marked elevation around 2 weeks after CO poisoning, peaking at around 30 days.

Table 2 Range and mean value of FA and ADC for each group

	FA		ADC ($\times 10^{-3}$ mm^2/s)	
	Range	Mean	Range	Mean
Group S	0.239–0.353	0.326 ± 0.040	0.494–0.622	0.551 ± 0.045
Group A	0.352–0.447	0.395 ± 0.029	0.487–0.601	0.517 ± 0.021
Controls	0.363–0.445	0.400 ± 0.027	0.472–0.580	0.517 ± 0.023

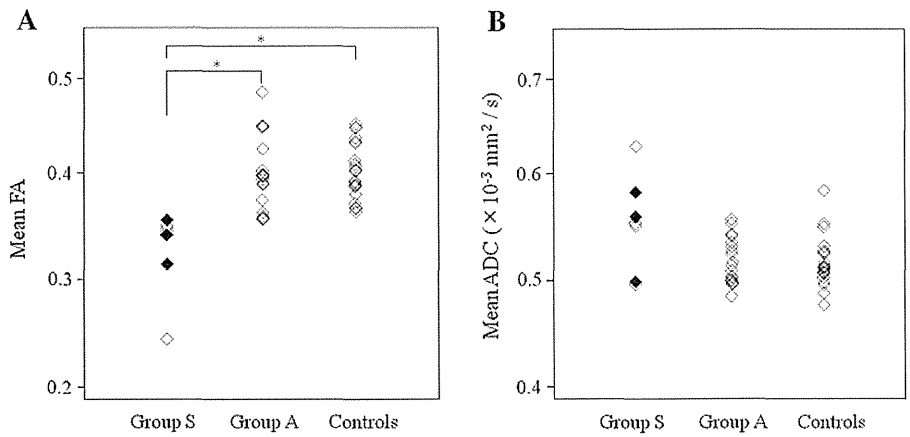

Fig. 2 Differences of mean FA (**a**) and mean ADC (**b**) values in the centrum semiovale bilaterally among group S, group A and controls. In group S, *black and white squares* represent patients with DNS and persistent symptoms, respectively (*$p < 0.001$).

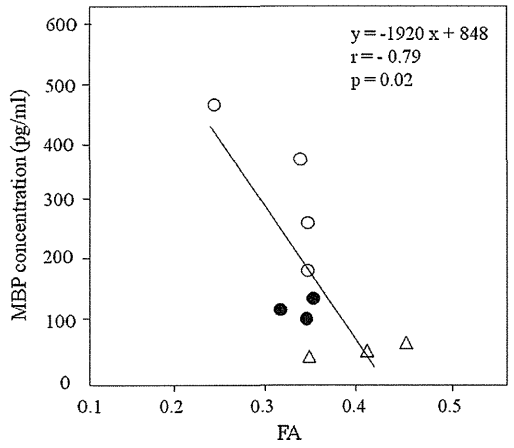

Fig. 3 Correlation between FA and MBP concentration in ten patients showing MBP concentration >40 pg/ml. *White circle*, patient with persistent chronic symptoms in group S; *black circle*, patient with DNS in group S; *triangle*, patient in group A

The timing for MBP measurements in the present study was thus established at 2 weeks (between day 12 and day 16) after admission. As a result, the incidence of abnormal MBP concentration was significantly higher in patients with chronic neuropsychiatry symptoms (group S) than in patients without chronic symptoms (group A) or controls. These results suggest that patients in group S certainly suffered from demyelinating changes somewhere in the CWM and support the theory that chronic neuropsychiatric symptoms after CO intoxication result from progressive demyelination in the CWM [24, 25]. The MBP concentration in case 6 was slightly lower (99 pg/ml) than the abnormal level, but the patient displayed akinetic mutism compatible with DNS at 1 week after measurement of MBP. The concentration of MBP in this patient might have been on the way to reaching abnormal levels, as demyelination of CWM in patients with DNS has been considered to undergo gradual progression during the lucid interval [13]. MBP concentrations of DNS patients were between those of patients with persistent symptoms in group S and those of patients in group A (Table 1; Fig. 3). These findings may indicate that demyelination begins to progress during the lucid interval before DNS. Although measuring MBP concentrations thus offers a useful indicator for assessing the extent of demyelination due to CO poisoning, detection of MBP using a lumbar tap is a highly invasive procedure and only indicates white-matter damage somewhere within the CNS.

Neuroimaging is minimally invasive and can visualize any region in the CWM. T2-weighted imaging (T2WI) often depicts abnormalities in the CWM in CO-poisoned patients. However, the interpretation of findings from routine MRI is difficult, as hyperintense foci in the CWM on T2WI can represent various progressive histological changes, including vasogenic edema, multiple necrosis, extensive axonal destruction and/or demyelination without axonal destruction [4, 12]. We therefore performed DTI in the same period as detection of MBP, since DTI is potentially more sensitive for assessing the extent of demyelinating changes in the CWM than other MRI sequences. As progressive reduction of FA values with age has been reported [5], we compared patients <60 years old with age-matched controls in this study. The finding of no significant difference in mean age among groups S, group A and controls suggests a negligible contribution of aging to FA values in this study. Previous reports have documented damage in various regions of the CWM after CO poisoning [8, 18, 28]. Indeed, some studies have reported correlations between FA values in various regions of the CWM in the chronic phase and cognitive dysfunction among CO-poisoned patients with DNS [16, 23, 30]. However, the centrum semiovale in the CWM has been suggested as a key region responsible for chronic neurological symptoms [4, 10, 19, 22]. A study using DTI at various phases after CO poisoning has also shown that FA in the centrum semiovale changes in parallel with cognitive impairments or neurological symptoms [17]. Based on these reports, we placed the ROI on the centrum semiovale to measure FA and ADC from DTI. As a result, mean FA for group S presenting with chronic neuropsychiatric symptoms was significantly lower than that for group A presenting with no chronic symptoms or that for controls consisting of healthy volunteers, whereas no significant difference was evident between group A and controls. In contrast, mean ADC did not differ significantly among the three groups. FA must be more sensitive for detecting CWM damage than ADC. Furthermore, these findings suggest that white matter fibers in the centrum semiovale were demyelinated in the subacute phase (2 weeks after poisoning) in CO-poisoned patients presenting with chronic symptoms. Notably, reductions in FA, suggestive of demyelination, were already present in the centrum semiovale before the recurrence of symptoms in the three patients with DNS. The reliability of this finding is supported by the result that MBP concentrations in DNS patients showed greater increases than those in group A patients at 2 weeks. These findings indicate the possibility of using FA in the centrum semiovale as an appropriate examination for predicting DNS during the lucid interval.

Our pilot study of DTI for CO-poisoned patients showed that FA enables representation of damage to white matter fibers in the centrum semiovale of patients with chronic neuropsychiatric symptoms [3]. That report, however, failed to demonstrate any correlation between FA in the centrum semiovale and MBP concentration, presumably because of the small sample size. Although subject criteria

were more strictly established in the present study than in our previous investigation, the greater number of subjects in this study allow us to show a linear correlation between FA and MBP in ten patients showing MBP concentrations >40 pg/ml. This finding validated the use of the centrum semiovale to represent various demyelinated lesions in the CWM, and FA in the centrum semiovale obviously offers a quantitative indicator of demyelination in CO-poisoned patients with chronic neuropsychiatric symptoms.

Some limitations must be considered in the interpretation of the study results. First, FA in the centrum semiovale may not strictly mirror the amount of demyelination in the whole CWM, although FA in the centrum semiovale correlated with MBP concentration. In group S, FA values in the centrum semiovale of the three DNS patients were not clearly different from those of the four patients with persistent symptoms (Fig. 2a), whereas MBP seemed to allow differentiation between subgroups in group S (around 100 pg/ml in patients with DNS and ≥150 pg/ml in patients with persistent chronic symptoms). This discrepancy might hypothetically be explained if demyelinated lesions in patients with persistent symptoms vary more than those in DNS patients. FA measured in this study suggests the magnitude of demyelination in the centrum semiovale, whereas MBP concentration not only indicates the magnitude, but also the width of demyelination in the whole CNS. We think that FA in the centrum semiovale cannot allow differentiation of the severity of CWM damage among subjects including patients with DNS and those with persistent symptoms. Second, the chronic neuropsychiatric symptoms seen after CO poisoning may not be solely attributable to demyelinating changes in fibers of the centrum semiovale. However, knowing to the focus on the region of the CWM is obviously very useful when evaluating the extent of CO-induced CWM damage using neuroimaging. We considered that the centrum semiovale represents the main region of damage and should be the focus of attention on neuroimaging in the subacute phase after CO poisoning [10]. Third, the sample size in this study was still small, with markedly fewer subjects in group S than in group A. The small number of DNS patients resulted in difficulties with statistical comparisons between subgroups in group S and other groups. However, the small sample size resulted from the strict entry criteria for this study. Furthermore, we did not select subjects with any bias other than the criteria established for this study. Indeed, percentages for patients with and without chronic symptoms in this study were in agreement with the results of previous reports [6, 33]. Fourth, findings in this study cannot be applied to patients over 60 years old. In senior patients, FA values may be overestimated as aging may lead to reduced FA values.

Conclusions

This is the first report to find that FA in the CWM correlates with MBP concentrations in the CSF during the subacute phase in CO-poisoned patients. The identification of a significant negative correlation between FA in the centrum semiovale and MBP concentration validates the concept that the centrum semiovale can reveal various demyelinated lesions in the CWM and that FA in the centrum semiovale offers a quantitative indicator of demyelination in CO-poisoned patients with chronic neuropsychiatric symptoms.

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Conflicts of interest None.

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DIAGNOSTIC NEURORADIOLOGY

Detecting damaged regions of cerebral white matter in the subacute phase after carbon monoxide poisoning using voxel-based analysis with diffusion tensor imaging

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Abstract
Introduction The present study aimed to detect the main regions of cerebral white matter (CWM) showing damage in the subacute phase for CO-poisoned patients with chronic neurological symptoms using voxel-based analysis (VBA) with diffusion tensor imaging (DTI).
Methods Subjects comprised 22 adult CO-poisoned patients and 16 age-matched healthy volunteers as controls. Patients were classified into patients with transient acute symptoms only (group A) and patients with chronic neurological symptoms (group S). In all patients, DTI covering the whole brain was performed with a 3.0-T magnetic resonance imaging system at 2 weeks after CO exposure. As procedures for VBA, all fractional anisotropy (FA) maps obtained from DTI were spatially normalized, and FA values for all voxels in the whole CWM on normalized FA maps were statistically compared among the two patient groups and controls.
Results Voxels with significant differences in FA were detected at various regions in comparisons between groups S and A and between group S and controls. In these comparisons, more voxels were detected in deep CWM, including the centrum semiovale, than in other regions. A few voxels were detected between group A and controls. Absolute FA values in the centrum semiovale were significantly lower in group S than in group A or controls.
Conclusions VBA demonstrated that CO-poisoned patients with chronic neurological symptoms had already suffered damage to various CWM regions in the subacute phase. In these regions, the centrum semiovale was suggested to be the main region damaged in the subacute phase after CO inhalation.

Keywords CO poisoning · Diffusion tensor imaging · Fractional anisotropy · Myelin basic protein · Voxel-based analysis

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Introduction

Among patients who survive acute poisoning, the majority show complete resolution of various acute neurological symptoms with inhalation of oxygen and/or exposure to hyperbaric oxygenation (HBO_2). However, approximately 30% of patients display chronic neurological symptoms due to late encephalopathy. Of these, approximately two thirds display persistent neurological symptoms and one third show delayed neuropsychiatric sequelae (DNS), which are recurrent neuropsychiatric symptoms after an interval of

apparent normality ("lucid interval", with a mean duration of 3 weeks) following apparent recovery from acute symptoms [1, 2]. Late encephalopathy causing chronic neurological symptoms including DNS has been recognized to result from progressive demyelination in the deep cerebral white matter (CWM) due to CO poisoning [3–5]. Strict examination of the extent of CWM damage in the early stage after CO intoxication may allow prediction of not only clinical outcomes for patients with persistent symptoms but also occurrence of DNS. Neuroimaging is suitable for such examination, enabling objective and non-invasive evaluation of brain damage.

Brain damage causing chronic neurological symptoms after CO poisoning has been rigorously investigated using neuroimaging. The common findings of magnetic resonance imaging (MRI) in CO-induced brain are appearance of lesions in any of the bilateral basal ganglia, such as, the globus pallidus, putamen, caudate nucleus, and thalamus [6–8]. However, chronic neurological symptoms due to CO poisoning cannot be explained solely by damage to these structures [2–4, 9, 10]. Many previous reports using conventional MRI at various phases have shown lesions widely dispersed throughout the CWM, such as, in the centrum semiovale, periventricular white matter, corpus callosum, or cerebellum and have documented causality between damage to the CWM and chronic neurological symptoms [10–14]. However, the main regions of CWM damaged in the early stage after CO intoxication have not yet been clarified. If the extent of CO-induced CWM damage is to be evaluated using neuroimaging, knowing the region of CWM to focus on is obviously very important. Determination of the main region showing damage in the early stage in patients with chronic symptoms thus becomes a cornerstone.

Diffusion tensor imaging (DTI), an MRI sequence, is potentially more sensitive for detecting demyelination in CWM when compared with conventional MRI [15, 16]. Among various quantitative parameters such as apparent diffusion coefficient (ADC) and eigenvalue derived from DTI, fractional anisotropy (FA) has been recognized as the most useful tool for evaluating the integrity of CWM fibers [17]. Indeed, FA is frequently used for evaluating the extent of damaged CWM fibers in patients with demyelinating diseases such as multiple sclerosis [18, 19]. FA represents a numerical value between 0 and 1 and decreases with increasing demyelination of CWM fibers. Use of FA is thus also adequate for determining the main regions of CWM showing damage in CO-poisoned patients. Previous studies have demonstrated correlations between FA values in various regions of CWM and cognitive dysfunction in CO-poisoned patients with DNS [14, 20, 21]. In those reports, FA value has been measured in a region of interest (ROI) manually placed in various regions on neuroimaging. However, placement of ROIs is limited to specified regions and is thus biased.

As voxel-based analysis (VBA) is a quantitative, unbiased, whole-brain method that can detect voxels showing statistically significant changes in any quantitative parameter on neuroimaging [22], this method offers advantages in searching for abnormalities throughout the entire brain. The aim of the present study was to detect the main regions of CWM damaged in the early stage among CO-poisoned patients with chronic neurological symptoms. Using VBA, we compared FA values in the subacute phase after CO intoxication in the entire CWM between CO-poisoned patients with and without chronic neurological symptoms.

Patients and methods

Patients

All study protocols were approved by the Ethics Committee of Iwate Medical University, Morioka, Japan. Patients admitted to Iwate Medical University Hospital between September 2008 and December 2010 and meeting the entry criteria were recruited to this study. Entry criteria for this study were: age, ≤60 and >20 years; diagnosis of CO poisoning caused by a fire or charcoal burning; no past history of brain disorders, including surgical operation, irradiation, stroke, infection, or demyelinating disease; and provision of written informed consent to participate. Diagnosis was based on a present history of exposure to CO and presence of acute neurological symptoms on admission. A total of 22 patients (20 men and two women; mean age, 42±12 years; range, 22–57 years) were enrolled. Mean duration from scene of CO exposure to arrival at our institute was 5.6 h (range, 0.5–81 h). We evaluated acute neurological symptoms including level of consciousness using the Glasgow Coma Scale and percentage of carboxyhemoglobin in arterial blood immediately after admission for each patient. All patients were treated using HBO_2 started within 24 h of admission and continued with a single daily session for a mean of 11.1 days (range, 1–60 days). The day of CO inhalation was defined as day 1 in this study. We subsequently observed neurological symptoms for 6 weeks after admission. General intellectual function was estimated using the mini-mental state examination (MMSE) [23] at 6 weeks (days 40–44) after CO exposure, by a trained psychiatrist (K.S.). We defined the normal range, borderline range, and dementia according to MMSE scores of ≥27, 26–22, and ≤21, respectively. When scores were within the borderline range, patients with educational background of ≥9 years and evidence of obvious personality change according to interviews with

the patient's family were diagnosed with dementia. Patients were assigned to one of two study groups according to clinical behaviors after CO poisoning: Group A, patients in whom acute neurological symptoms improved completely and who showed no neurological symptoms at 6 weeks; and group S, patients with chronic neurological symptoms at 6 weeks. An age-matched control group of 16 healthy volunteers (14 men and two women; mean age, 41± 11 years; range, 22–58 years) was established for comparison with patient groups.

Measurement of myelin basic protein

To evaluate the extent of demyelination in CWM, myelin basic protein (MBP) concentration in cerebrospinal fluid (CSF) was examined using lumbar tap between days 12 and 16 in all CO-poisoned patients. Normal and abnormal ranges for MBP concentration were defined as <102 and ≥102 pg/ml, respectively. When the level of MBP was below the limit of detection, the result from the laboratory was reported as MBP of ≤40 pg/ml. Frequencies of abnormal MBP concentration (≥102 pg/ml) between groups S and A were compared using Fisher's exact probability test. In this analysis, statistical significance was established at the $p<0.05$ level.

VBA from DTI

DTI covering the entire brain was performed for all patients at 2 weeks (between days 12 and 16), using a 3.0-T MRI scanner (Signa Excite HD; GE Healthcare, Milwaukee, WI, USA) and an eight-channel head coil. Pulse sequences were as follows: axial single-shot, spin-echo, echo-planar imaging (EPI); repetition time, 10,000 ms; echo time, 66 ms; six motion-probing gradient directions (b value=1,000 s/mm^2); matrix size, 128×128; field of view, 24×24 cm; slice thickness, 4.0 mm with 1.5-mm interslice gaps; number of slices, 24; number of excitations, 3; parallel imaging reduction factor, 2; and acquisition time, 3 min and 40 s. For all normal volunteers, DTI was performed using the same procedures as described above. We generated FA maps from the DTI dataset for all patients and controls. FA maps were transferred to a workstation and transformed from DICOM image format into Analyze image format using free software MRIcro (http://www.cabiatl.com/mricro/) [24].

VBA was performed to compare FA for all voxels in the FA map (which was limited to the CWM) between the two patient groups and controls. VBA in this study was undertaken as described below, according to methods outlined in a previous report [25]. VBA was performed using software developed by one author (S.F.), and Statistical Parametric Mapping version 5 (SPM5), which is freely available and distributed at the Wellcome Trust Centre for Neuroimaging Web page (http://www.fil.ion.ucl.ac.uk/spm/). First, we produced a white-matter FA map (Fig. 1a) for each patient, using extracted voxels corresponding to CWM. Second, we performed spatial normalization for all individual white-matter FA maps, to enable comparison of white-matter FA maps showing different sizes of the brain among groups. Spatial normalization was performed by co-registering the individual white-matter FA map to an FA template (Fig. 1b), which was created from white-matter FA maps of the 16 healthy volunteers. To create the FA template, white-matter FA maps of the 16 healthy volunteers were co-registered to an EPI template provided from SPM5. These co-registered white-matter FA maps were smoothed using a 12-mm isotropic Gaussian kernel. An averaged image obtained from all smoothed co-registered white-matter FA maps

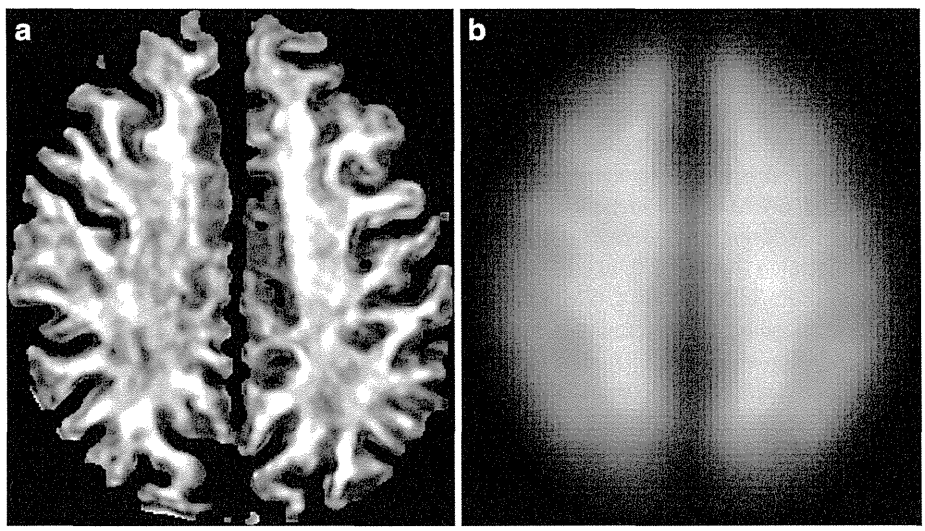

Fig. 1 Images for procedure of VBA. **a** White-matter FA map extracted from FA map. **b** FA template created from white-matter FA maps of 16 healthy volunteers

from healthy volunteers was defined as the FA template and comprised 97,537 voxels, each with a size of 2×2×2 mm. Third, the co-registered FA map for each patient was created by co-registering individual white-matter FA maps to the FA template. All co-registered FA maps were also smoothed with a 12-mm isotropic Gaussian kernel, to improve the validity of statistical inferences and to reduce inter-individual variation due to co-registration procedures. Finally, FA values in all voxels on the co-registered FA map were compared voxel-by-voxel among the two patient groups and controls. In analyses in our VBA, the p value was established as 0.01 in the Mann–Whitney U test for two-group comparisons. As comparison was performed in three pairs among three groups, the significance threshold was equivalent to $p<0.03$, according to Bonferroni correction [26]. Voxels showing a significant difference with value of $p<0.03$ were identified as reddish-colored voxels on the FA template (Fig. 2a–c).

Measurement of absolute FA and ADC

To verify the reliability of VBA, absolute FA was measured for all subjects, using an ROI placed on the region depicting the greatest numbers (the widest area) of voxels showing a significant difference. The ROI was manually placed in this region on non-diffusion-weighted images, and FA values were automatically calculated using MRIcro. We measured ADC values using the same procedure applied for measuring FA. Absolute FA and ADC for each subject were determined as the mean of values measured twice by the same investigator (S.F.). The second measurement was performed 1 week after the first test, using a different randomized order of measurements from the first test. We then calculated mean FA and ADC of the right and left sides for each subject. Mean values of absolute FA and ADC for right and left sides were compared between groups using the Mann–Whitney U test with Bonferroni correction. In this comparisons, statistical significance was established at the $p<0.03$ level. Intra-operator reliability for all absolute FA and ADC values were evaluated according to classification of the intra-class correlation coefficient (ICC) [27]. For ICC(1,1) and ICC(1,k) as intra-operator reliability, agreement of all absolute values between the first and second tests was analyzed for right and left lesions, using one-factor analysis of variance. We analyzed correlations between absolute FA and MBP in seven patients in groups S, using Spearman's correlation coefficient by rank test.

T2-weighted image

T2-weighted imaging (T2WI) was performed at the same time as performance of DTI, using a 3.0-T MRI scanner and an eight-channel head coil. Pulse sequences were as follows: axial fast spin-echo imaging; repetition time, 3,000 ms; echo time, 83 ms; matrix size, 512×256; field of view, 24×24 cm; slice thickness, 4.0 mm with 1.5-mm interslice gaps; number of slices, 24; number of excitations, 1; parallel imaging reduction factor, 2; and acquisition time, 2 min and 36 s. We observed findings of hyperintense foci in the globus pallidus and CWM. Any region in CWM was defined as the same region where absolute FA was measured. Frequencies of hyperintense foci in the globus pallidus and CWM were compared between groups S and A using Fisher's exact test.

Results

Clinical data for all patients are summarized in Table 1. Among the 22 patients, seven patients (including three patients with DNS) displayed chronic neurological symptoms at 6 weeks after CO inhalation and were defined as group S. All DNS occurred after performances of MRI and MBP measurements. In the remaining 15 patients, acute symptoms resolved completely within 3 days after admission, and no neurological symptoms were indentified after 6 weeks. These patients were defined as group A. Abnormal MBP concentrations (≥102 pg/ml) were detected in six of seven patients in group S (the remaining one patient showed detectable levels, 102>MBP≥41 pg/ml, within the normal range), whereas all patient in group A showed normal MBP concentrations (only one patient showed detectable levels). Frequencies of abnormal MBP level differed significantly between groups S and A ($p<0.001$).

We successfully obtained DTI data with adequate image quality in all subjects. In comparisons of VBA between group A and controls, a few areas of accumulated voxels with significant differences in FA were seen in CWM on the FA template (Fig. 2a). In contrast, many areas of voxels with significant difference were observed at various regions in the CWM between group S and controls and between groups S and A (Fig. 2b, c). Notably, the number of voxels showing significant difference was greater in the region corresponding to deep CWM, including the centrum semiovale, than in other regions, in comparisons both between group S and controls and between groups S and A. No voxels with significant difference were seen in the brainstem or cerebellar peduncles in any comparisons.

Absolute FA was measured bilaterally in the centrum semiovale (Fig. 3), and mean FA for the right and left sides was compared among groups. Mean absolute FA in the centrum semiovale of both sides ranged from 0.24 to 0.35 (mean±standard deviation, 0.33±0.04) in group S, ranged from 0.36 to 0.44 (mean, 0.40±0.02) in group A, and

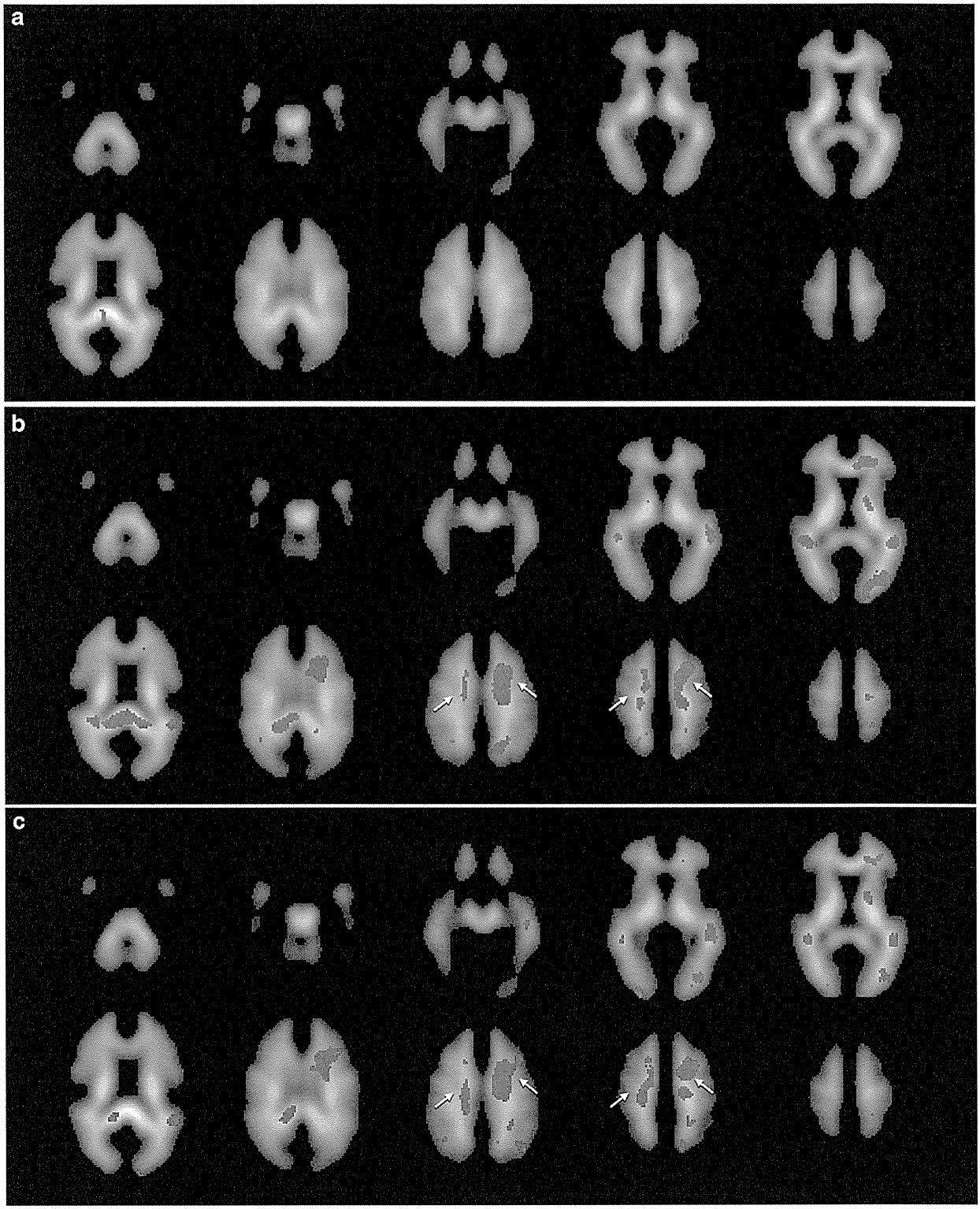

Fig. 2 Demonstrations of FA templates showing *voxels* with significant difference in FA values. **a** Comparison between group A and controls. **b** Comparison between group S and controls. **c** Comparison between groups S and A. *Arrows* indicate the region of the centrum semiovale

Table 1 Summary of CO-poisoned patients

	No.	Age (year)/sex	COHb (%)	GCS	Symptoms on admission	Symptoms at 6 weeks	MMSE at 6 weeks	MBP (pg/ml)	T2WI	
									GP	CS
Group S	1	29/M	24.8	6	IC	Dementia	23	252	+	+
	2	57/M	25.1	10	IC	Parkinsonism	27	176	+	−
	3	38/M	1.5	6	IC	Apallic syndrome	NS	468	−	+
	4	56/M	39.7	3	IC	Dementia	16	376	+	+
	5	55/M	13.5	14	Headache	Akinetic mutism[a]	NS	99	+	−
	6	29/M	3.6	14	Headache	Parkinsonism[a]	28	130	+	−
	7	46/M	28.6	6	IC	Dementia[a]	23	110	+	+
Group A	8	57/M	33.3	10	IC	None	30	<40	+	−
	9	44/F	13.7	15	Dizziness	None	30	<40	−	−
	10	26/M	1.9	15	Dizziness	None	30	<40	−	−
	11	47/M	17.2	13	Dizziness	None	30	<40	−	−
	12	28/M	19.2	15	Headache	None	30	<40	−	−
	13	41/F	2.7	11	IC	None	30	<40	−	+
	14	22/M	20.5	15	Headache	None	29	52.8	−	−
	15	55/M	14.0	14	Dizziness	None	30	<40	−	−
	16	35/M	25.3	7	IC	None	30	<40	−	−
	17	56/M	12.2	15	Headache	None	30	<40	−	−
	18	36/M	31.0	12	Headache	None	30	<40	−	−
	19	34/M	44.1	10	IC	None	30	<40	−	−
	20	57/M	40.1	11	Dizziness	None	30	<40	−	−
	21	32/M	19.3	12	Headache	None	30	<40	−	−
	22	34/M	38.6	5	IC	None	30	<40	+	−

COHb carboxyhemoglobin, *GCS* Glasgow Coma Scale, *MMSE* mini-mental state examination, *MBP* myelin basic protein, *IC* impairment of consciousness, *NS* no study performed due to unconsciousness, *T2WI* T2-weighted imaging, *GP* globus pallidus, *CS* centrum semiovale

[a] Delayed neuropsychiatric sequelae

ranged from 0.36 to 0.45 (mean, 0.40±0.03) in controls. Mean absolute FA was significantly lower for group S than for group A or controls (p=0.0006), whereas no significant difference was found between group A and controls (Fig. 4a). A weak correlation appeared to exist between FA and MBP (r=−0.56), but no significant correlation was found (p=0.18). Mean absolute ADC at the centrum semiovale ranged from 0.49 to 0.62 (mean, 0.55±0.05) in group S, ranged from 0.49 to 0.55 (mean, 0.51±0.02) in group A, and ranged from 0.47 to 0.58 (mean, 0.52±0.02) in the controls. No significant difference was found in comparisons for absolute ADC between the three groups (Fig. 4b). Intra-operator reliability for absolute FA was classified as "almost perfect" for the centrum semiovale bilaterally; ICC(1,1) and ICC(1,k) were 0.88 and 0.93 for the right side and 0.95 and 0.98 for the left side, respectively. Intra-operator reliability for absolute ADC was also classified as "almost perfect" for bilateral centrum semiovale; ICC(1,1) and ICC(1,k) were 0.98 and 0.99 for the right side and 0.91 and 0.95 for the left side, respectively. Frequency of hyperintensity on T2WI differed significantly between groups S and A in both the globus pallidus (p=0.002) and centrum semiovale (p=0.021). The p value in the comparison of groups S and A was lower for absolute FA than for T2WI.

Discussion

Previous studies have led to the establishment of the theory that degeneration of CWM causing chronic neuropsychiatric symptoms after CO poisoning results from demyelination due to CO-mediated toxicity. Alterations in levels of MBP, a major myelin component in the central nervous system (CNS), following lipid peroxidation are considered to lead to reversible auto-immunological demyelination of CNS [3, 4]. Inflammation associating with demyelination leads to further damages to the CWM [5]. Based on these theories, the concentration of MBP in CSF has recently been proposed as a marker of demyelination in CWM after CO intoxication [28]. In that report, MBP concentration in patients with DNS was markedly elevated around 2 weeks

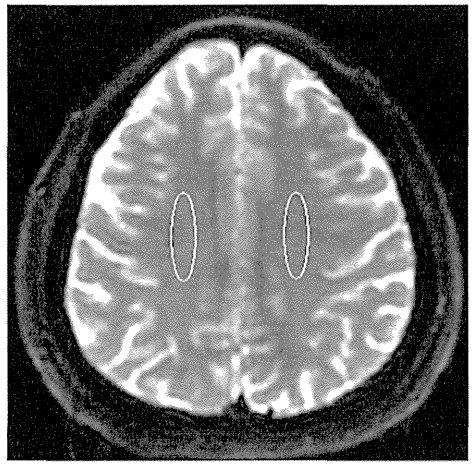

Fig. 3 ROIs were placed at the centrum semiovale bilaterally on non-diffusion-weighted imaging (b=0 s/mm^2). *Circles* show ROIs

after admission and afterward changed in parallel with symptoms. The timing for both MBP measurements and performance of MRI in this study was thus established around 2 weeks after CO inhalation. No patient presented with abnormal MBP concentration (≥102 pg/ml) in group A, whereas almost all patients (excluding case 5 in Table 1) in group S showed abnormal levels. MBP concentration in case 5 was slightly lower (99 pg/ml) than the abnormal level, but the patient displayed akinetic mutism compatible with DNS at 1 week after measurement of MBP. MBP concentration in this patient might have been on the way to reaching abnormal levels, as demyelination of CWM in patients with DNS has been considered to undergo gradual progression during the lucid interval [29, 30]. Frequency of abnormal MBP level differed significantly between groups S and A. These results indicate that patients of group S certainly suffered from demyelination of CWM at 2 weeks after CO inhalation. Furthermore, high frequency of hyperintense foci on T2WI in the globus pallidus in group S suggests that patients in group S certainly suffered from severe cerebral hypoxia due to CO inhalation, as ischemia or necrosis in the globus pallidus, a specific characteristic of severe CO poisoning, is caused by a hypoxic effect of CO exposure [31].

Although measuring MBP concentrations is a useful method for estimating the extent of demyelination due to CO poisoning, detection of MBP using lumbar tap is a highly invasive procedure and only indicates white-matter damage somewhere in the total CNS. MRI shows low invasiveness and can be used to visualize any region in the CWM. If the extent of CWM damage is to be evaluated in the early stage after CO poisoning using MRI, the region to be observed on neuroimaging is very important. Some previous reports of conventional MRI findings at various phases after CO poisoning have suggested the centrum semiovale in the CWM as a key region responsible for chronic neurological symptoms [10, 32, 33]. A study using DTI at various phases has also shown that FA in the centrum semiovale changes in parallel with cognitive impairments or neurological symptoms [34]. Based on those reports, we have previously reported that decreased FA from DTI and changes in metabolites from magnetic resonance spectroscopy (MRS) in the centrum semiovale at 2 weeks reflect the extent of demyelination due to CO poisoning and also offer predictors of DNS occurrence [29, 35]. However, the main region of CWM that is damaged in the subacute phase has remained unclear, as no previous studies analyzing damage to the entire CWM in the early stage after CO poisoning have been reported. The present study used VBA to detect regions showing significant

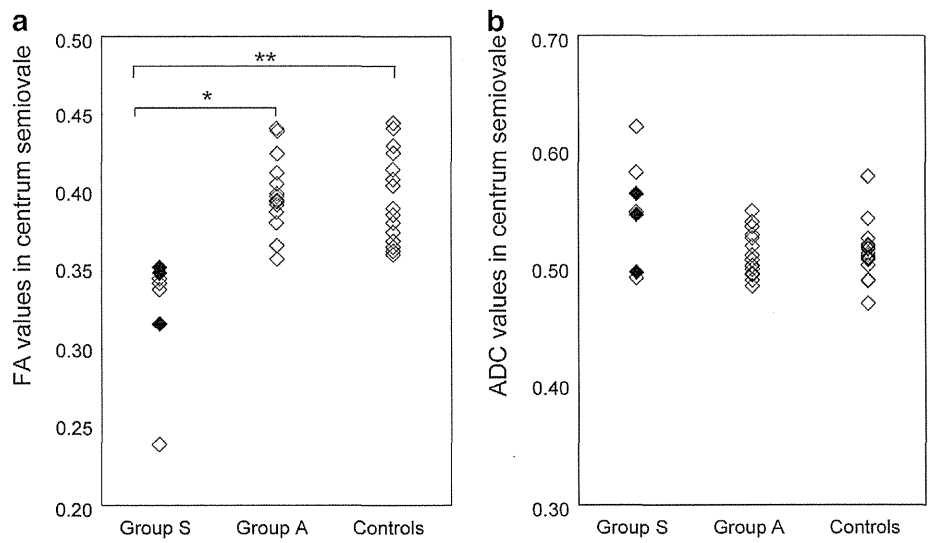

Fig. 4 Absolute FA (**a**) and ADC (**b**) values in the centrum semiovale bilaterally. In group S, *black* and *white squares* represent patients with DNS and persistent symptoms, respectively. *p=0.0006; **p=0.0005

changes in FA due to demyelination from whole CWM at 2 weeks after CO exposure in patients with chronic neurological symptoms. As a result, a few voxels with significant differences in FA were observed in a comparison between group A and controls. This finding might be negligible, as group A patients showed no chronic symptoms and no elevation of MBP concentration. In contrast, voxels with significant differences in FA were detected at various regions in the CWM between group S and controls and between groups S and A. These results suggest that severe damage had already occurred in various regions in the CWM by 2 weeks after CO inhalation in patients subsequently presenting with chronic neurological symptoms. Notably, a range of voxels corresponding to deep CWM, including the centrum semiovale, was detected most widely in both comparisons between groups S and A and between group S and controls. These results indicate that patients with chronic neurological symptoms suffer widespread damage around the centrum semiovale. We considered that the centrum semiovale represents the main region of damage and should be the focus of attention on neuroimaging in the subacute phase after CO poisoning. When measurements of FA in DTI and metabolites in MRS are performed using a single ROI in the subacute phase after CO poisoning, placement of the ROI on the centrum semiovale is appropriate to evaluate the extent of demyelination causing chronic neurological symptoms. To the best of our knowledge, only two previous studies of VBA for FA values in CO-poisoned patients have been reported, both from the same institute [36, 37]. Those reports have documented results from comparisons of FA in the CWM between different phases after CO poisoning. The present study is the first report of VBA in which FA values in CWM in the subacute phase after CO exposure were compared between patients who subsequently displayed chronic symptoms and those who did not.

As brain atrophy due to aging may influence FA [38, 39], we compared patients ≤60 years old with age-matched controls in this study. Although pathological brain atrophy caused by CO poisoning has been reported to present from 3 months after CO exposure [36], we performed all neuroimaging at 2 weeks after poisoning. The influences of brain atrophy on FA were thus negligible in this study. The absolute FA values in the centum semiovale for healthy volunteers in this study were similar to the range of 0.382–0.420 reported in previous studies [34, 40], and ICCs for ROI measurements showed an "almost-perfect" agreement. These results confirm the reliability of FA values in the present study. Results showing that mean absolute FA for both sides of the centrum semiovale was significantly lower for Group S than for Group A and controls support the reliability of VBA in this study. In contrast, no significant differences were found in comparisons of mean absolute ADC between the three groups. These results support the idea that FA is more useful than ADC for evaluating damage of CWM fibers. The finding that hyperintensity at the centrum semiovale on T2WI differed significantly between groups S and A might also support results of VBA in this study that the centum semiovale was severely damaged in group S patients. However, the p value in statistical comparison between groups S and A was lower for absolute FA value (p=0.0006) than for frequency of hyperintensity on T2WI (p=0.021). This result suggests the validity of using FA for estimating the extent of damages in the centrum semiovale after CO poisoning.

Some limitations regarding the interpretation of results in this study must be considered. First, we demonstrated differences in FA between groups, but did not examine relationships between different regions of damage and symptoms. We thus could not verify the hypothesis that the centrum semiovale represents a key region responsible for chronic neurological symptoms after CO poisoning. Symptoms in some patients in group S, for whom absolute FA in the centrum semiovale did not strongly decrease (Fig. 4), might have resulted from damage to in regions other than the centrum semiovale. Further study of relationships between different damaged regions and symptoms in the CO-poisoned patient are needed. Second, the number of patients in this study was small. We thus could not classify group S patients to subgroups consisting of patients with persistent symptoms and patients with DNS. However, Fig. 4 demonstrates that absolute FA in the centrum semiovale did not differ greatly between patients with persistent symptoms and those with DNS. A weak correlation appeared to exist between FA and MBP, but no significant correlation was found. One reason for this result might be the small sample size of the study. Further investigation of a larger number of symptomatic patients is needed. Third, we performed VBA using FA maps calculated from thick-slice DTI with an interslice gap, which results in a partial volume effect. Although we thought VBA limited to the CWM in the present study would decrease the partial volume effect, there is the possibility that a partial volume effect could lead to underestimation of absolute FA values in the centrum semiovale, which consists of crossing fibers. Further investigation using high-resolution isotropic-voxel DTI may be needed.

Conclusion

The present study demonstrated that deep CWM including the centrum semiovale was the main region of CWM damaged in the subacute phase in a majority of patients with chronic neurological symptoms.

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Conflict of interest We declare that we have no conflict of interest.

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