compression and to examine the influence of chronic sympathetic nerve activation on acute ischemic stroke.

METHODS

Subjects and study design

This study was conducted in consecutive patients with acute ischemic stroke admitted between April 2008 and March 2010 to Hiroshima University Hospital within 24 h of stroke onset. Exclusion criteria included the inability to undergo magnetic resonance imaging (MRI) examination, the administration of thrombolysis, an National Institutes of Health Stroke Scale (NIHSS) score $\geqslant$23 on admission, the use of anti-hypertensive or vasopressor medicines within 72 h after admission, and surgery within 72 h after admission. In addition, we excluded patients with medullary infarctions, which might have effects on RVLM. The stroke subtype was determined based on MRI findings, electrocardiography, and carotid artery and cardiac ultrasound findings by at least two stroke specialists according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.[15] The acute treatment was determined based on the stroke subtype in accordance with the established guidelines. Neurological severity was evaluated using the NIHSS upon admission and 14 days after admission.[16] Their clinical outcome was evaluated with the modified Rankin scale at the time of discharge.[17] The study was approved by the institutional review board of Hiroshima University Hospital.

Hypertension was defined as the use of any anti-hypertensive medicines before admission or a confirmed blood pressure of $\geqslant$140/90 mm Hg at rest after 2 weeks from stroke onset. Diabetes mellitus was defined as HbA1c $\geqslant$6.5%, fasting blood sugar $\geqslant$126 mg dl^{-1}, or the use of any anti-diabetic medicines. Hyperlipidemia was defined as total cholesterol $\geqslant$220 mg dl^{-1}, low-density lipoprotein cholesterol $\geqslant$140 mg dl^{-1} on admission, or the use of any anti-hyperlipidemic medications. For 72 h after admission, blood pressure and pulse rate were measured every 6 h on the unaffected arm in a resting supine position using a standard automated sphygmomanometer without any anti-hypertensive medicines. Blood pressure and pulse rate variability was evaluated with successive variation (SV), defined as the square root of the averaged squared differences between two successive measurements.[18]

The presence or absence of RVLM vascular compression was evaluated on a 3T MRI unit (GE, Fairfield, CT, USA) using 3D time-of-flight (slice thickness of 1.0 mm, TR/TE/flip angle, 23/3.4/18°). The RVLM was located at the root-entry zone of cranial nerves IX and X (Figure 1). The location was defined as follows: upper and lower borders of the root-entry zone were determined by the uppermost and lowest fibers of the IX/X nerve bundle entering the medulla; the anterior border of the root-entry zone was defined as the transition of the olivary convexity to the concavity of the retro-olivary sulcus; and the posterolateral border was located at the junction of parenchymal brain tissue and individual nerve fibers.[2] Arterial compression (vertebral artery or posterior-inferior cerebellar artery) within this defined area was considered 'positive' for RVLM, and patients with no arterial compression were considered 'negative.' We defined arterial compression as deformation of the medulla surface. Therefore, instances in which the artery just contacted the surface of the medulla were excluded from the criteria for RVLM vascular compression. The presence of RVLM vascular compression was evaluated by two neurologists who were unaware of each patient's medical history.

Statistical analysis

Data are shown as the mean ± s.d. or median (minimum-maximum) for continuous variables. Statistical analysis for comparison of the two groups was performed using Student's *t*-test or the Mann–Whitney *U*-test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables. *P*-values $<$0.05 were considered statistically significant. Receiver operating characteristic curves were configured to establish cut-off points for SV that optimally predicted RVLM compression.

RESULTS

Of the 86 enrolled patients, 11 did not undergo MRI, 4 had missing data, 3 received thrombolysis, 4 had an NIHSS score $\geqslant$23 on admission, 5 received anti-hypertensive or vasopressor medicines, and 3 had surgery within 48 h of hospital admission. Thus, all analyses were performed on the remaining 56 patients. MRI was performed at a mean time of 32.3 h after symptom onset (range, 4–50 h). Table 1 shows the baseline characteristics of all patients. Vascular compression on the RVLM was identified in 15 (26.8%) patients (hereafter, referred to as the compression group). Age was not significantly different between the patients with and without RVLM vascular compression. In the compression group, the proportion of males was significantly higher (93.3 *vs.* 63.4%, *P*=0.03). The prevalence of hypertension was significantly higher in the compression group (93.3 *vs.* 61.0%, *P*=0.02). At the time of admission, there was no significant difference

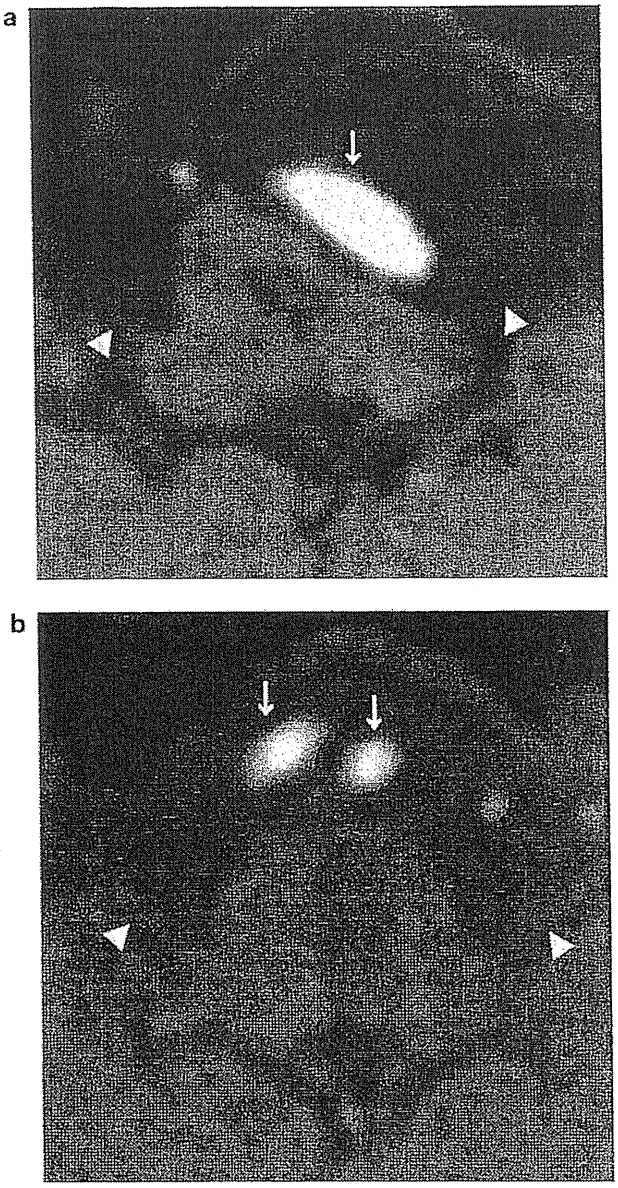

Figure 1 Axial 3D time-of-flight (TOF) images are shown (a) compression case, (b) non-compression case. The RVLM extends in a craniocaudal direction from the root entry zone of cranial nerve IX down to the upper part of cranial nerve XI. The RVLM is dorsolateral to the olive and medioventral to the root entry zone of cranial nerves IX and X (arrowhead). The compression of vertebral artery (arrow) or posterior-inferior cerebellar artery within this defined area was considered 'positive' for RVLM.

Table 1 Baseline characteristics, blood pressure and NIHSS on admission, and ischemic stroke subtypes

Variables	*Compression (n=15)*	*Non-compression (n=41)*	P-*value*
Age, mean years ± s.d.	71.6 ± 9.37	73.2 ± 9.5	0.59
Male, n (%)	14 (93.3%)	26 (63.4%)	0.03
Risk factors			
Hypertension, n (%)	14 (93.3%)	25 (61.0%)	0.02
Diabetes mellitus, n (%)	9 (60.0%)	17 (41.5%)	0.22
Hyperlipidemia, n (%)	7 (46.7%)	27 (65.9%)	0.19
Baseline NIHSS	4 (0–8)	4 (0–20)	0.29
Baseline SBP, mean mm Hg ± s.d.	159.1 ± 8.9	149.5 ± 26.1	0.17
Baseline DBP, mean mm Hg ± s.d.	81.1 ± 14.7	75.4 ± 15.2	0.88
Ischemic stroke subtype			
Atherothrombotic, n (%)	5 (33.3%)	13 (31.7%)	
Cardioembolic, n (%)	2 (13.3%)	17 (41.5%)	
Small vessel, n (%)	3 (20.0%)	5 (12.2%)	0.17
Other etiology, n (%)	5 (33.3%)	6 (14.6%)	

Abbreviations: DBP, diastolic blood pressure; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure.

Table 2 Relationship between RVLM vascular compression and clinical improvement at 14 days

	Improved (NIHSS ⩾4 improvement or NIHSS=0)	*Unimproved*	*OR (95% CI) probability*
Compression	4 (26.7%)	11 (73.3%)	0.21 (0.06–0.78)
Non-Compression	26 (63.4%)	15 (36.6%)	*P*=0.01

Abbreviations: CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; RVLM, rostral ventrolateral medulla.

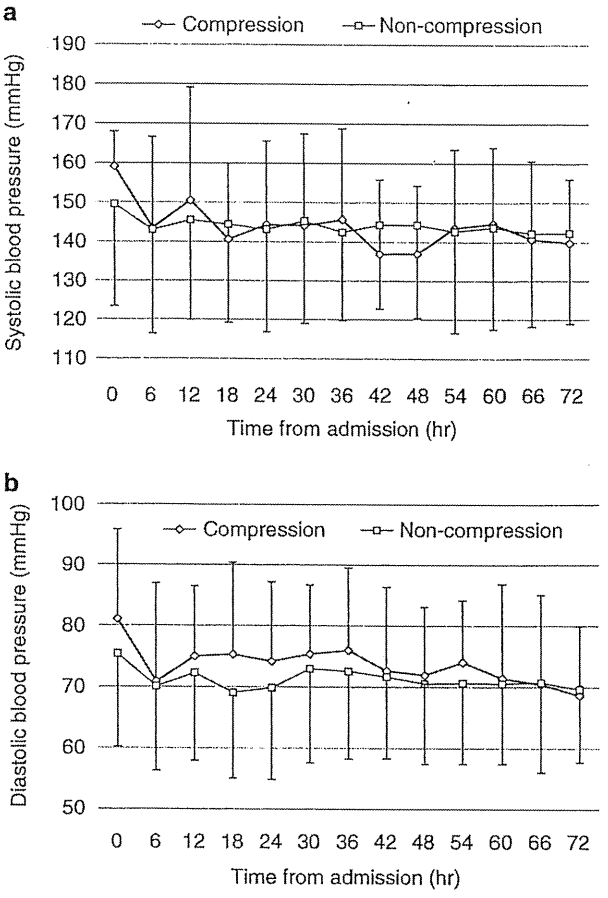

Figure 2 Average blood pressures during 72 h of hospitalization in the compression group (n=15) and the non-compression group (n=41). (a) Systolic blood pressure, (b) diastolic blood pressure.

in NIHSS scores, and no significant difference was observed in systolic or diastolic blood pressure between the groups. In addition, the subtype of ischemic stroke was not significantly different between the groups (P=0.17).

The patients were defined as clinically improved when their NIHSS score decreased more than four points or recovered to 0 on day 14 (ref. 19). The proportion of clinically improved patients was 26.7% in the compression group and 63.4% in the non-compression group (Table 2), with a significantly lower rate of improvement in the compression group (odds ratio, 0.21 (95% CI=0.06–0.78); P=0.01).

Figures 2 and 3 show the systolic and diastolic blood pressures and the SV values of the patients up to 72 h after admission. At all time points, no significant differences were observed in mean systolic or diastolic blood pressures between the two groups. However, the SV value of the systolic blood pressure was significantly higher in the compression group during the first 24 h after admission (P<0.0001) and during the 72 h period after admission (P<0.0001). Conversely, there were no significant differences in the diastolic blood pressure SV value between the groups at any time point. The pulse rate SV value was significantly higher in the compression group during the 72 h period after admission (8.7 ± 2.7 b.p.m. *vs.* 7.3 ± 2.2 b.p.m., P<0.05)

In the explanatory analysis, the best cutoff SV value of the systolic blood pressure during the 72 h period after admission obtained from the Receiver operating characteristic curve was 15.3 mm Hg, which predicted RVLM compression with a sensitivity of 67% and a specificity of 88% (area under the curve=0.783; P=0.0002). In the compression group, the patients with SV <15.3 mm Hg tended to show a more favorable outcome, defined using modified Rankin scale, than the patients with SV ⩾15.3 mm Hg (P=0.10) (Figure 4).

DISCUSSION

This study found that blood pressure variability during the acute ischemic stroke phase was significantly greater in patients with RVLM vascular compression than in those without. In addition, decreased improvement in neurologic symptoms was observed in patients with RVLM vascular compression compared with patients without RVLM vascular compression. In the compression group, patients with high SV values were more likely to have unfavorable outcomes. The association between blood pressure variability and acute ischemic stroke outcome has previously been reported.[12,13] To our knowledge, this study is the first to clearly show an association between RVLM vascular compression, blood pressure variability and functional prognosis.

It has been reported that a large proportion of patients with RVLM vascular compression have hypertension.[1–4] Mechanical stimulation of the RVLM causes glutamate release from RVLM vasomotor neurons, thereby increasing sympathetic nerve activity.[20] RVLM vascular compression was observed in 7–22.2% of healthy individuals without

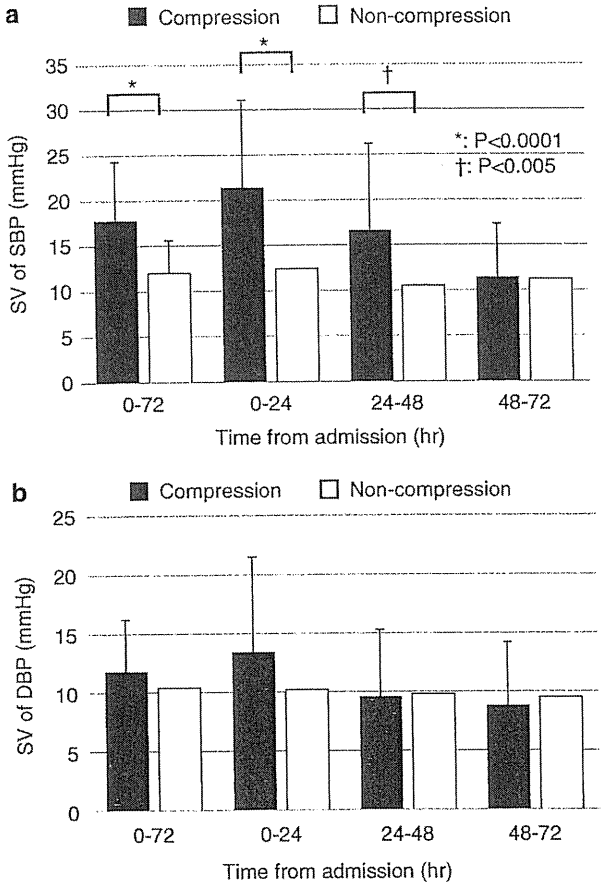

Figure 3 Successive variation (SV) of blood pressure during 72 h of hospitalization in the compression group (n=15) and the non-compression group (n=41). (a) Systolic blood pressure (SBP), (b) diastolic blood pressure (DBP).

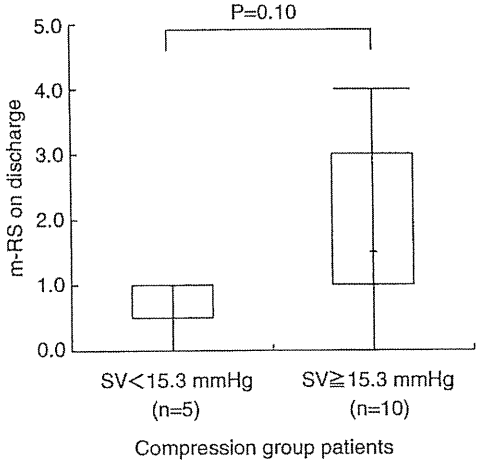

Figure 4 Box plot showing median modified Rankin scale (m-RS) on discharge (solid bar), interquartile range (bar width), and minimum/maximum values across SV < 15.3 mm Hg or ⩾15.3 mm Hg in compression group.

hypertension and in 74–90% of patients with hypertension.[3,4,21,22] Our results demonstrated that the prevalence of hypertension was 93.3% (14/15) in patients with RVLM vascular compression, and it was higher than that in patients without RVLM vascular compression, which is consistent with previous studies. The prevalence of hypertension was 60.5% (25/41) in patients without RVLM vascular compression, which is similar to other previous population-based studies of ischemic stroke.[23,24] We propose that RVLM vascular compression is one of the important factors related to hypertension. However, it is still unknown whether RVLM vascular compression is the cause of hypertension because no study has prospectively followed young normotensive subjects with RVLM vascular compression to evaluate the incidence of hypertension. Conversely, several reports have demonstrated that surgical decompression of the RVLM reduced sympathetic nerve activity and normalized systemic blood pressure.[5,6] In addition, in our study, the patient with RVLM vascular compression who did not show hypertension (n=1) had higher blood pressure variability than patients without RVLM vascular compression and had a poor prognosis on discharge. In the other target organs (the heart), it has been reported that the regression of left ventricular hypertrophy depends on not only blood pressure levels, but also the reduction of cardiac sympathetic drive.[25] Therefore, we think that chronic sympathetic nerve activation influences target organ damage even when patients are not hypertensive.

In the present study, the compression group had a significantly higher SV value and a significantly lower proportion of clinically improved patients. In the compression group, the sympathetic nerve may be chronically activated. Hypertension is likely one of the markers of this chronic sympathetic nerve activation, which explains the significantly higher rate of hypertension in the compression group. In patients with chronic hypertension, cerebral blood flow decreases rapidly with cerebral ischemia in association with an increasing cerebral oxygen extraction fraction.[26] Therefore, blood pressure variability may change cerebral blood flow. This pathological response may deteriorate the improvement of neurological symptoms in patients with RVLM vascular compression.

Our findings demonstrated that the difference in blood pressure between the groups was not significant, although it tended to be slightly higher in the compression group. Interestingly, the variability in blood pressure was significantly greater in the compression group. The sympathetic nervous system is generally persistently activated during acute ischemic stroke, leading to increased blood pressure regardless of the presence or absence of RVLM vascular compression. Therefore, it is difficult to detect a difference in blood pressure with or without RVLM vascular compression during the acute ischemic stroke phase. By contrast, RVLM vascular compression clearly activates the sympathetic nervous system, leading to increased burst-like firing of sympathetic nerves,[27] which is thought to have a role in blood pressure variability.

It has been reported that patients with RVLM vascular compression have increased sympathetic nerve activity.[8] In addition, the increased sympathetic nerve activity may induce high blood pressure variability because it was reduced after ganglion blockade with trimethaphan.[28] In parallel to this response, when sympathetic nerve activity increases, the baroreflex counteracts this activation in normal subjects. However, it is still unknown whether the increase in sympathetic nerve activity is caused by increased sympathetic nerve traffic, increased vascular sensitivity, or impaired baroreflex buffering in patients with RVLM vascular compression. It has been reported that baroreflex buffering and baroreflex-mediated vasopressin release are severely impaired in patients with RVLM vascular compression.[7] When the impaired baroreflex is associated with RVLM vascular compression, blood pressure variability may increase with increased sympathetic nerve activity. It has been suggested that restoration of baroreflex sensitivity may prevent stroke in the animal model.[29] It is possible that the

restoration of baroreflex sensitivity reduced blood pressure variability and, therefore, stroke incidence. However, blood pressure variability was not examined in the experiment by Liu *et al.* Therefore, it is unknown whether stroke prevention with the restoration of baroreflex sensitivity was mediated by the reduction of blood pressure variability. In our study, we evaluated the association of blood pressure variability at the acute ischemic stroke phase and its prognosis for patients with or without RVLM vascular compression. However, we were unable to evaluate blood pressure variability at the pre-ischemic stroke period. Therefore, further studies are needed to clarify the association between blood pressure variability and the occurrence of stroke in patients with RVLM vascular compression.

Various measures of variability of individual blood pressure profiles have been used. The most common measures of variations are the extreme values, such as maximum, minimum, range (difference between maximum and minimum), s.d., or coefficient of variation (s.d. over mean).[30] In the present study, we selected SV as a parameter of within-patient blood pressure variability. This parameter includes the serial variation on a time sequence, whereas other measures, such as s.d. and coefficient of variation, ignore the sequential nature of such a data set. Therefore, when analyzing the sequential nature of data, as in the present study, it is better to use SV. If not, time-invariant measures, such as s.d. or coefficient of variation, can result in a misleading prediction of prognosis and is less informative for blood pressure management.

In the present study, we evaluated RVLM vascular compression with MRI images. However, it is possible that cases in which there appeared to be RVLM vascular compression had a low degree of compression that did not result in sympathetic nerve activation. Thus, we examined the association between SV and prognosis in the compression group, which was divided into two groups using the best cutoff SV value of the systolic blood pressure during the 72 h period after admission. As a result, the patients with high SV values tended to show more unfavorable prognosis than the patients with low SV values. Therefore, large-scale studies are required to confirm the influence of the SV value on the compression group prognosis.

The present study has several limitations. First, a selection bias may exist because of the small sample size at a single institution. Therefore, it will be necessary to conduct a similar multi-center study to confirm whether these results can be generalized. In addition, our results may not reflect all ischemic stroke patients because of the frequent exclusion of severe cases. It is highly possible that the severe cases have poor prognoses regardless of the presence of RVLM vascular compression or that the influence of RVLM vascular compression might be low. To study these issues, we are planning a multi-center, large-scale trial to define the association of RVLM vascular compression with the prognosis of ischemic stroke patients. Another limitation of the current study is that we did not examine the correlation between RVLM vascular compression and SV value with the use of anti-hypertensive medications before stroke onset. In particular, medications that inhibit sympathetic nerve activation may influence blood pressure and blood pressure variability. However, the half-lives of these anti-hypertensive medications are not more than 24 h, and their effects decrease with time. In our study, the SV value for blood pressure variability was calculated at 72 h after admission. Therefore, pre-medication with anti-hypertensives should have had a limited effect on the SV value. Finally, the correlation with ischemic location was not fully investigated. A previous study demonstrated that patients with infarctions involving the insular cortex tended to suffer from autonomic dysfunction,[31] which may influence blood pressure. However, in the present study, few patients had infarctions involving the insular cortex, and the proportion of patients with infarctions involving the insular cortex was similar between the compression and non-compression groups (two in the compression group (13.3%) and five in the non-compression group (12.2%)). Thus, the influence of ischemic location in the present study may also be limited.

In conclusion, this study found that patients with RVLM vascular compression had greater variability in blood pressure during the acute ischemic stroke phase, which may be a factor related to poorer prognosis. In the future, larger scale prospective studies are required to confirm the influence of RVLM vascular compression in acute ischemic stroke.

ACKNOWLEDGEMENTS

This study was supported in part by research grants from the Ministry of Health, Labor and Welfare, Japan.

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potential candidates for the optimum surgical technique in the current guidelines.

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1. Prevention of Intracerebral Hemorrhage

Recommendations

1. Treating hypertension is the most vital step to reduce the risk of intracerebral hemorrhage (ICH) (Grade A).
2. People should be encouraged to take a moderate amount of vegetables and fruits every day (Grade B).

Recommendations, *continued*

3. Heavy alcohol consumption leading to abnormal blood γ-GTP levels should be discouraged (Grade B).
4. In the case of hypocholesterolemia, it has generally been agreed that the its underlying hepatic disease and coexisting hypertension should be treated. Lowering serum cholesterol level with statins does not increase the incidence of ICH, but some data have implied that intervention for stroke patients may increase the recurrence of ICH (Grade B).
5. We recommend the very careful consideration of an appropriate dose of each antithrombotic drug and its dual medications, that are required to control concurrent hypertension (Grade B).

Evidence

The prevention of ICH consists of collective intervention for the improvement of lifestyle habits, primary prevention in a high-risk group such as hypertensives and secondary prevention in stroke patients. Adherence of individual patients to identified risk factor reduction is a prerequisite for the prevention of ICH which has high morbidity and mortality rates.

The higher the blood pressure, the higher the incidence of ICH. Hypertension is a risk factor to which the utmost attention should be paid for Asian people in whom ICH occurs more frequently than in Caucasians[14,15] (IIb). A meta-analysis has demonstrated the usefulness of antihypertensive therapy for the prevention of stroke and recurrent stroke[16] (Ia). The Perindopril Protection against Recurrent Stroke Study (PROGRESS) showed that an antihypertensive regimen decreased the incidence of ICH by half for over 3.9 years of follow-up in 6105 patients with previous cerebrovascular events[17] (Ib). A surge of blood pressure in the early morning has been identified to be an independent risk factor for ICH[18] (IIa); thus, to control hypertension strictly all day long is the most important action to reduce the risk of ICH.

A meta-analysis of epidemiologic surveys demonstrated that the incidence of stroke decreased in a group with a high consumption of green and yellow vegetables and fruits[19] (IIb). In Japanese people, the incidence of ICH was lower when the general population were in the habit of eating fruits and vegetables every day[20] (IIb).

It is generally agreed that heavy alcohol consumption increases blood pressure and causes hepatic dysfunction thereby decreasing the serum levels of blood coagulation factors and cholesterol, thus elevating the risk of ICH[21,22] (IIb). In a group with increased γ-GTP levels, which are an index of hepatic dysfunction associated with heavy alcohol consumption, the incidence of ICH increased regardless of blood pressure or lipid levels[23] (IIb).

There was indeed a concern that hypocholesterolemia raised the risk factor for ICH, but it has been shown that the hazard arises only when low cholesterol levels are combined with hypertension[23] (IIb). It is generally agreed to treat concurrent hypertension with hypocholesterolemia, although there are no clinical data regarding any

relationship between the attenuation of serum cholesterol and the lower incidence of ICH.

A meta-analysis showed that statin treatment for improving hyperlipidemia did not increase the incidence of ICH[24] (Ia). In contrast, analyses including the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study showed that improving the lipid profile in patients with previous stroke elevated the incidence of ICH[25,26] (Ia). No correlation was observed between levels of LDL cholesterol and the occurrence of ICH[27] (IIb). It is generally agreed that lipid-improving statins should be administered with care to male, elderly, hypertensive and chiefly hemorrhagic stroke patients, and that their physicians have been educated as to the need for blood pressure reduction as a feasible countermeasure[28] (IIb).

Antithrombotic therapy increases frequency of ICH onset and the subsequent hematoma expansion. Whenever 2 different antiplatelet drugs are administered or an antiplatelet is combined with an anticoagulant over the long term, it is generally agreed that the clinicians of such patients should weigh the advantage of the antithrombotic actions against the disadvantage of bleeding complications[29] [*See also additional remarks for the English version]. The appropriate adjustment of the blood anticoagulation intensity and uninterrupted stabilization of blood pressure with antihypertensive medication are recommended[30,31] [*See also additional remarks for the English version] (Ia).

Not only chronic renal failure requiring hemodialysis[32] but also chronic kidney disease with a low glomerular filtration rate (GFR) elevate the risk of ICH[33] (IIa). A subanalysis of the PROGRESS reported that antihypertensive therapy with an angiotensin-converting enzyme inhibitor prevented recurrent stroke in renal disease patients,[34] but they have no available data regarding how renal protection works well against ICH.

Diabetes mellitus (DM) increased the incidence of stroke; type 1 DM elevated the incidence of ICH, whereas type 2 DM did not[35] (IIa). The treatment of DM with hypoglycemic drugs, such as insulin and insulin-resistance improving drugs, has not been reported to prevent ICH.[36] Concurrent hypertension remains the most important target[37] (Ib).

Asymptomatic cerebral microbleeds detected in T2*-weighed MRI in patients with acute cerebral infarction are a risk factor for stroke recurrence, particularly symptomatic ICH for Japanese people[38,39] (IIa). No research on the efficacy of antihypertensive therapy and safety of antithrombotic therapy in patients with hemorrhage-prone microangiopathy is currently under way.

No data are available to help develop preventive measures specific to non-hypertensive ICH associated with cerebral amyloid angiopathy that frequently occurs and recurs in the late elderly.

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***Additional remarks for the English version**

A Japanese prospective observational study of 4009 patients who were taking antithrombotic agents for stroke and cardiovascular disease provided us with the information that adding an antiplatelet agent to either another antiplatelet or warfarin increased the events of life-threatening major bleeding, including intracranial hemorrhage, over a 19-month follow-up.[40] An increase in blood pressure during antithrombotic medication was positively associated with the occurrence of hemorrhagic stroke; the optimal cutoff was 130/81 mmHg to predict imminent danger.[41]

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2. Nonsurgical Treatment of Hypertensive Intracerebral Hemorrhage

2-1. Administration of a hemostatic

Recommendations

1. When there is no abnormality with the blood coagulation system in patients with a normal hypertensive intracerebral hemorrhage (ICH) in the acute phase, administration of blood products including blood coagulation factors is not recommendable (Grade C2).
2. Even if the ICH is the hypertensive type, administration of blood products such as platelets, prothrombin complex and fresh frozen plasma should be considered for patients with concurrent abnormal platelets or blood coagulation system and a bleeding tendency according to their clinical conditions (Grade C1).
3. There is no adequate scientific evidence supporting the use of capillary stabilizers or antiplasmin agents for the treatment of acute ICH (Grade C1).

Evidence

The usefulness of the recombinant activated coagulation factor VIIa (rFVIIa) for acute ICH was evaluated first in 2 small-scale studies[42,43] and a medium-scale study.[44] In the latter medium-scale study, 399 patients with ICH within 3 hours after onset were randomized into a placebo or rFVIIa group. The results showed that rFVIIa treatment significantly inhibited increases in the bleeding volume, decreased mortality, and improved the dysfunction level at 90 days after onset (Ib). In the subsequent Phase III, large-scale study,[45] 841 patients were treated with rFVIIa or a placebo within 4 hours after onset in a randomized, double-blind manner. Compared with the placebo, the rFVIIa treatment significantly inhibited increases in ICH and improved the level of functional care and the level of neurological disorder by 15 days after hemorrhage, whereas the primary endpoint defined as the frequency of deaths and serious sequelae (mRS score 5-6) at day 90 did not improve (Ib).

Even if the main cause of ICH is hypertension, administration of blood products such as platelets, prothrombin complex and fresh frozen plasma should be considered for patients with concurrent abnormal platelet or blood coagulation system and a bleeding tendency according to individual clinical conditions.[46,47]

There is no large-scale clinical study on the use of capillary stabilizers or antiplasmin agents in patients with acute ICH as compared with a placebo. Only reports on a small-scale controlled study[48] and use of these drugs without comparators[49] are available[50] (IIb-III).

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INTERNAL MEDICINE

□ CASE REPORT □

Cerebral Sinus Thrombosis and Heparin-induced Thrombocytopenia in a Patient with Paroxysmal Nocturnal Hemoglobinuria

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Abstract

Paroxysmal nocturnal hemoglobinuria is a rare acquired disorder of clonal hematopoietic stem cells and it is characterized as a hypercoagulable disorder. We report a 36-year-old woman with the rare triad of paroxysmal nocturnal hemoglobinuria, cerebral sinus thrombosis triggered by infection, and rapid-onset heparin-induced thrombocytopenia after resensitization of heparin. This case raises caution for heparin-induced thrombocytopenia in paroxysmal nocturnal hemoglobinuria.

Key words: cerebral venous thrombosis, stroke in young adults, hematology

(Inter Med 49: 941-943, 2010)
(DOI: 10.2169/internalmedicine.49.3053)

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia because of clonal hematopoietic stem cells and is characterized as arterial and venous thrombosis (1). Heparin-induced thrombocytopenia (HIT) is caused by platelet factor 4/heparin-reactive autoantibody. Heparin treatment causes HIT in 0.1-5% of patients (2). We present a rare case with hemorrhagic stroke during heparin treatment for cerebral venous thrombosis in PNH.

Case Report

A 36-year-old Japanese woman with PNH had been treated with warfarin for relapsing mesenteric thrombosis, but voluntarily stopped treatment medication for four months.

She had been diagnosed as PNH at the age of 31 during pregnancy by hemolytic anemia, positive Ham test, and CD 59 negative neutrophils and CD14 negative monocytes. She was admitted to our hospital because of abdominal pain, a fever of 38.5℃, a left-sided headache and vomiting. Laboratory data showed white blood cell count for $18.12\times10^3/mm^3$, CRP for 13.36 mg/dL, antinuclear antibody for × 80. Single-strand DNA, double-strand DNA, Lupus anticoagulant, and anticardiolipin antibodies were negative. Aseptic meningitis was diagnosed by painful herpes-like scalp dermatitis, neck stiffness, and cerebrospinal fluid findings (clear yellow liquid with 230 mmH_2O of pressure, a total cell count of $41\times10^6/L$, 41 polycytes and 14 monocytes, a total protein concentration of 2.05 g/L, and a glucose concentration of 0.56 g/L) with no bacterial culture. She was treated with acyclovir and cefepime.

She had no neurological deficits on admission, and the results of a head CT scan were normal. Although the patient's headache and nausea gradually improved, she developed dysphasia and right hemiparesis 5 days after admission. CT and MRI of the head showed intracerebral hemorrhage in the left temporal lobe, edema surrounding the left lobe of the cerebellum, and mild mastoiditis and parotitis on the left side. MRI revealed thrombus in the left transverse sinus. The left transverse and sigmoid sinuses were not detected by MR venography (Fig. 1). The results of MR angiography

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Received for publication October 21, 2009; Accepted for publication January 28, 2010
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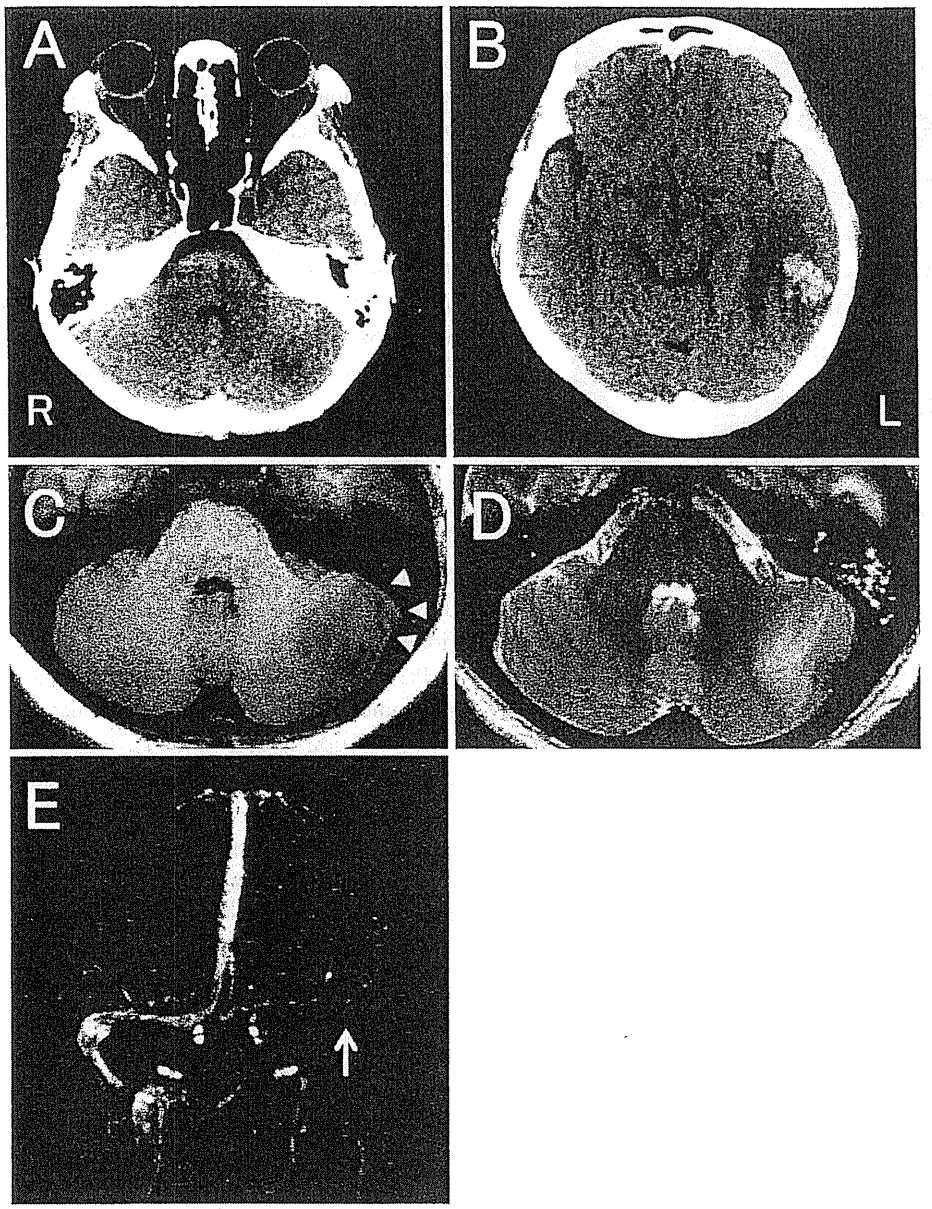

Figure 1. CT indicates intracerebral hemorrhage in the left temporal lobe, edema surrounding the left lobe of the cerebellum (A, B). T1- (C) and T2- (D) weighted images detect sinus thrombosis (arrowheads) and mild mastoiditis on the left side. Hemorrhagic change exists in the left cerebellar hemisphere. The left transverse and sigmoid sinuses are not detected by MR venography (E, arrow).

and carotid ultrasonography were normal. Venous sinus thrombosis was diagnosed, and unfractionated heparin was started at a dose of 10,000 units/day. The patient's symptoms gradually improved after the heparin injection. On the 13th day after admission, heparin treatment was temporarily stopped for the second cerebrospinal tap. After heparin treatment was restarted, the patient complained of dizziness, headache, and vomiting, which led to somnolence 11 hours later. A CT scan showed cerebellar vermis hemorrhage and hydrocephalus (Fig. 2). Heparin treatment was stopped, and cerebral decompression was performed. The platelet count decreased from 119×10^9/L to 60×10^9/L just before surgery. 4 Ts pretest clinical score (3) was as high as 6, included thrombocytopenia (2 points), clear onset on the 8 days after heparin exposure (2 points), progressive or recurrent thrombosis (1 point), and possible other cause for thrombocytopenia such as PNH (1 point). Then, we diagnosed the patient as clinical HIT. The patient died of brain herniation 17 days after admission. Autopsy was not conducted according to the family's request.

Discussion

Both HIT and bone marrow failure with PNH cause thrombocytopenia. There are a few reports of simultaneous coexistence of PNH and HIT (4, 5). Phosphatidyl serine on the hemolytic red blood cells which activates glycosylphosphatidylinositol anchored protein (GPI-AP) deficient

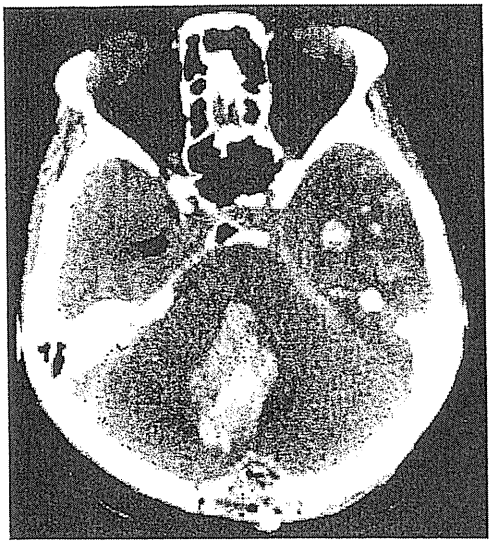

Figure 2. Cerebellar virmis hemorrhage is evident by CT.

platelets, anexcess of soluble urokinase receptor which inhibits fibrinolytic system, and large PNH granulocytes are considered to be risks of thromboembolic event in PNH (6, 7). The patient in our study showed 90% of GPI-AP-deficient granulocytes and repeated thrombotic events.

The reasons for acute worsening of symptoms and thrombocytopenia might be sinus thrombosis progression, infection, disseminated intravascular coagulation (DIC), and HIT. Intracranial or craniocervical infection is one of the reasons for sinus thrombosis. Sinus thrombus near the site of mastoiditis detected by MRI suggests that mastoiditis and parotitis extended to meningitis and caused the inflammatory response. Infection partially affected the worsening of symptoms, but did not account for acute thrombocytopenia. Severe infection sometimes causes thrombocytopenia and DIC, but the fever and CRP were improving. PNH also causes chronic thrombocytopenia, but seldom acute thrombocytopenia. Anti-HIT antibody by enzyme-linked immunoassay (ELISA) was negative in this patient. Serotonin release assay which is most selective test for HIT cannot be done in Japan. The false-negative proportion of anti-HIT antibody by ELISA is about 20% (8). Patients with the 4Ts score of more than 5 are diagnosed with HIT over 80% accuracy (9, 10). The score in this case was as high as 6, therefore we diagnosed her as clinical HIT.

HIT induces both arterial and venous thrombosis by activated platelets and endothelial cells. Platelet activation induces platelet consumption and thrombin formation causes hypercoagulation. We considered that the excess of sinus thrombosis caused by HIT blocked venous return and deteriorated cerebellar hemorrhage.

Patients previously exposed to heparin and having HIT antibodies sometimes have a rapid decrease of platelets after resensitization of heparin (11). Restart of heparin treatment after discontinuation for lumber puncture might have activated autoimmunity to platelets and caused a sudden episode of thrombocytopenia.

A major complication with PNH patients is thrombosis, and the causes of mortality are thrombosis (32%), infection (17%), and cerebral hemorrhage (8%) (1). Anticoagulation is needed to treat thrombosis, and we must keep in mind that thrombocytopenia caused by HIT.

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ORIGINAL RESEARCH

Positional Relationship between Recurrent Intracerebral Hemorrhage/Lacunar Infarction and Previously Detected Microbleeds

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BACKGROUND AND PURPOSE: Although MBs, ICH, and LI are secondary to cerebral microangiopathy, it remains unclear whether the location of subsequent ICH/LI corresponds to the previous location of MBs. We performed this study to clarify the positional relationship between recurrent ICH/LI and previously detected MBs.

MATERIALS AND METHODS: We evaluated patients with recurrent ICH/LI who had MBs, as shown on prior T2*-weighted MR imaging. We assessed retrospectively whether the location of recurrent ICH/LI corresponded to that of the prior MB. Patients with ICH were divided into the deep ICH group and the lobar ICH group, and the positional relationship between hematoma and previously detected MBs was evaluated.

RESULTS: A total of 55 patients, including 34 with recurrent ICH and 21 with recurrent LI were evaluated. Although the location of the LI corresponded to prior MBs in only 1 patient (4.8%), the location of ICH corresponded to prior locations of MBs in 21 patients (61.8%) (OR, 32.3; 95% CI, 3.86–270.3; $P < .001$). Among the patients with ICH, the correspondence ratio was higher in the deep ICH group (19 of 24 patients, 79.2%) than in the lobar ICH group (2 of 10 patients, 20%) (OR, 15.2; 95% CI, 2.42–95.3; $P < .002$).

CONCLUSIONS: The close positional association between recurrent ICH and prior MBs suggests that MBs represent hemorrhage-prone microangiopathy. In addition, different correspondence ratios between the deep ICH group and the lobar ICH group may be attributable to their different pathogenesis.

ABBREVIATIONS: ATBI = atherothrombotic brain infarction; CAA = cerebral amyloid angiopathy; CE = cardioembolic infarction; CI = confidence interval; DWI = diffusion-weighted imaging; ICH = intracerebral hemorrhage; LI = lacunar infarction; MB = microbleed; OR = odds ratio

MBs present as homogeneous round lesions with signal-intensity loss on gradient-echo T2*-weighted MR images. Pathologically, they represent hemosiderin deposits,[1,2] associated with small-vessel disease.

Previous studies have shown that MBs are observed more frequently in patients with ICH compared with patients with ischemic stroke.[3,4] Among patients with ischemic stroke, they are observed more frequently in patients with LI, which is based on small-vessel disease, compared with patients with ATBI or CE.[5,6] In addition, MBs are more prevalent among patients with recurrent stroke compared with patients with their first stroke.[4] Previous studies have also shown that the presence of MBs is an important risk factor for the occurrence of subsequent stroke, particularly hemorrhagic stroke.[7-9]

The topologic association, however, between the location of MBs and that of subsequent stroke is poorly understood. Although previous reports described the association between the hematoma and the distribution of MBs at the onset of ICH,[10,11] a few case reports[12,13] and several cases described in a prospective study that was performed for other purposes[7,14] have reported that the subsequent ICH occurred in the same lesion in which prior MBs were detected. Moreover, to our knowledge, topologic association in patients with LI has not been reported.

This retrospective study was designed to clarify the positional association between recurrent ICH/LI and previously detected MBs in a relatively large number of patients.

Materials and Methods

Study Design and Patients

We evaluated consecutive patients with acute recurrent ICH/LI who were admitted to our hospital from June 2003 to June 2008. Among them, the patients who had asymptomatic MBs identified on 1.5T gradient-echo T2*-weighted MR imaging, which was performed at the time of the prior stroke event, were included in the study. Patients with CE, ATBI, or undetermined classification were excluded. The diagnosis of acute stroke was made on the basis of neurologic and neuroradiologic examinations. Recurrent stroke was classified into ischemic stroke and ICH, and ischemic stroke was further subclassified as ATBI, CE, and LI, according to the diagnostic criteria based on the National Institute of Neurologic Disorders and Stroke Ad Hoc Committee Classification of Cerebrovascular Disease III.[15] Of the 55 patients included, 34 had recurrent ICH and 21 had recurrent LI.

The location of recurrent ICH was assessed by using CT, and the location of recurrent LI was assessed by DWI and apparent diffusion

Received October 28, 2009; accepted after revision February 12, 2010

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DOI 10.3174/ajnr.A2100

Table 1: Characteristics of patients with ICH and LI

Characteristic	ICH (n = 34)	LI (n = 21)	P Value[a]
Demographic data			
Median age, (yr) (range)	69.5 (51–84)	72 (57–89)	.020
Male sex, No. (%)	25 (73.5)	13 (61.9)	.365
Vascular risk factors			
Hypertension (%)	97.0	100	1.000
Diabetes mellitus (%)	24.2	31.6	.746
Hyperlipidemia (%)	45.2	52.6	.608
Antithrombotic therapy (%)	56.3	78.9	.135
Prior stroke subtype, No. (%)			
ICH	13 (38.2)	2 (9.5)	.029
LI	12 (35.3)	18 (85.7)	.005
ATBI	4 (11.8)	1 (5.9)	.639
CE	5 (14.7)	0 (0)	.144
No. of MBs, median (range)	12.5 (1–73)	6 (1–83)	.070
Time from prior stroke, median day (range)	247.5 (14–1873)	179 (3–860)	.188
Correspondence to MBs, No. (%)	21 (61.8)	1 (4.8)	<.001

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

coefficient maps. We assessed, retrospectively, whether the location of recurrent ICH/LI corresponded to that of the previously detected MBs. Furthermore, patients with ICH were divided into the deep ICH group (hematoma present in the thalamus, the putamen, the pons, and the cerebellum) and the lobar ICH group (hematoma in a subcortical location), and the positional relationship between the hematoma and previously detected MBs was evaluated. There were no patients with a recurrent caudate hemorrhage in the present study. Previous antithrombotic therapy, the number of previously detected MBs, and the duration from the prior stroke to the recurrence were also evaluated in each patient. In patients with recurrent ICH, the hemorrhage volume was also evaluated. The study protocol for the chart review was approved by our institutional review board.

Vascular Risk Factors

We assessed vascular risk factors such as history of previous stroke and the presence of hypertension, diabetes mellitus, or hyperlipidemia. "Hypertension" was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg, which were measured with an automated cuff-oscillometric device at least 2 times in the outpatient department before recurrence of stroke, or current medical treatment for hypertension. "Diabetes mellitus" was defined as a glycosylated hemoglobin A_{1c} concentration of ≥6.5% or current use of hypoglycemic agents. "Hyperlipidemia" was defined as a low-attenuation lipoprotein cholesterol level of ≥140 mg/dL or current cholesterol-lowering therapy. We also recorded the prevalence of antithrombotic therapy before occurrence of the recurrent stroke in each patient.

Neuroradiologic Examinations

All patients were examined by using a 1.5T clinical MR imaging unit (Magnetom Symphony; Siemens, Erlangen, Germany) with a section thickness of 5 mm and a 1.5-mm gap between sections. We used axial T2*-weighted gradient-echo sequences (TR/TE, 800/26 ms; flip angle, 20°; FOV, 230 × 230; matrix, 192 × 256) to detect MBs at the onset of the prior stroke. In addition, at the onset of the recurrent stroke, we also performed axial DWI with single-shot echo-planar spin-echo sequences (TR/TE, 5300/135 ms; FOV, 196 × 261; matrix, 80 × 128; b-values, 0 and 1000/mm^2) to evaluate the location of recurrent LI, and we performed axial head CT to evaluate the location and the volume of recurrent ICH. MBs were defined as homogeneous round lesions with a diameter of ≤5 mm characterized by signal-intensity loss on T2*-weighted MR images. Signal-intensity-loss lesions in the globus pallidum (which likely represented calcification) and the subarachnoid space (which likely represented adjacent pial vessels) were excluded. Intracerebral lesions were also excluded if they had a hemorrhagic component associated with tumor, arteriovenous malformation, cavernous hemangioma, or trauma.

"Corresponding" or "correspondence" was used if the location of MBs detected on prior T2*-weighted MR imaging was involved in the ICH detected on CT or the LI detected on DWI at the onset of recurrent stroke. Two of the authors (Y.S., H.N.) without detailed knowledge of the patients' clinical profiles retrospectively compared the same section of each film and determined the correspondence of MBs with subsequent stroke. In addition, we calculated the hemorrhage volume with the ABC/2 method, in which A is the greatest diameter on the largest hemorrhage section, B is the diameter perpendicular to A, and C is the approximate number of axial sections with hemorrhage multiplied by the section thickness.[16]

Statistical Analysis

For the cases of recurrent ICH versus LI and deep brain versus lobar ICH, the χ^2 test or Fisher exact test for independence was used for comparison of sex ratio, hypertension, diabetes mellitus, hyperlipidemia, antithrombotic therapy, and correspondence between prior MBs and recurrent stroke for each group. The Student t test was used for comparison of age at the time of recurrent stroke. The Mann-Whitney U test was used for comparison of the hemorrhage volume, the number of previously detected MBs, and the time from prior stroke to the recurrence in each ICH group. $P < .05$ was considered significant. The Statistical Package for the Social Sciences, Version 16.0 for Windows (SPSS, Chicago, Illinois) was used for statistical analysis.

Results

Baseline Data

Of the 55 patients included in this study, 34 had recurrent ICH (25 men and 9 women) and 21 patients had recurrent LI (13 men and 8 women). The patients with ICH (median age, 69.5 years; range, 51–84 years) were younger compared with the patients with LI (median age, 72 years; range, 57–89 years;

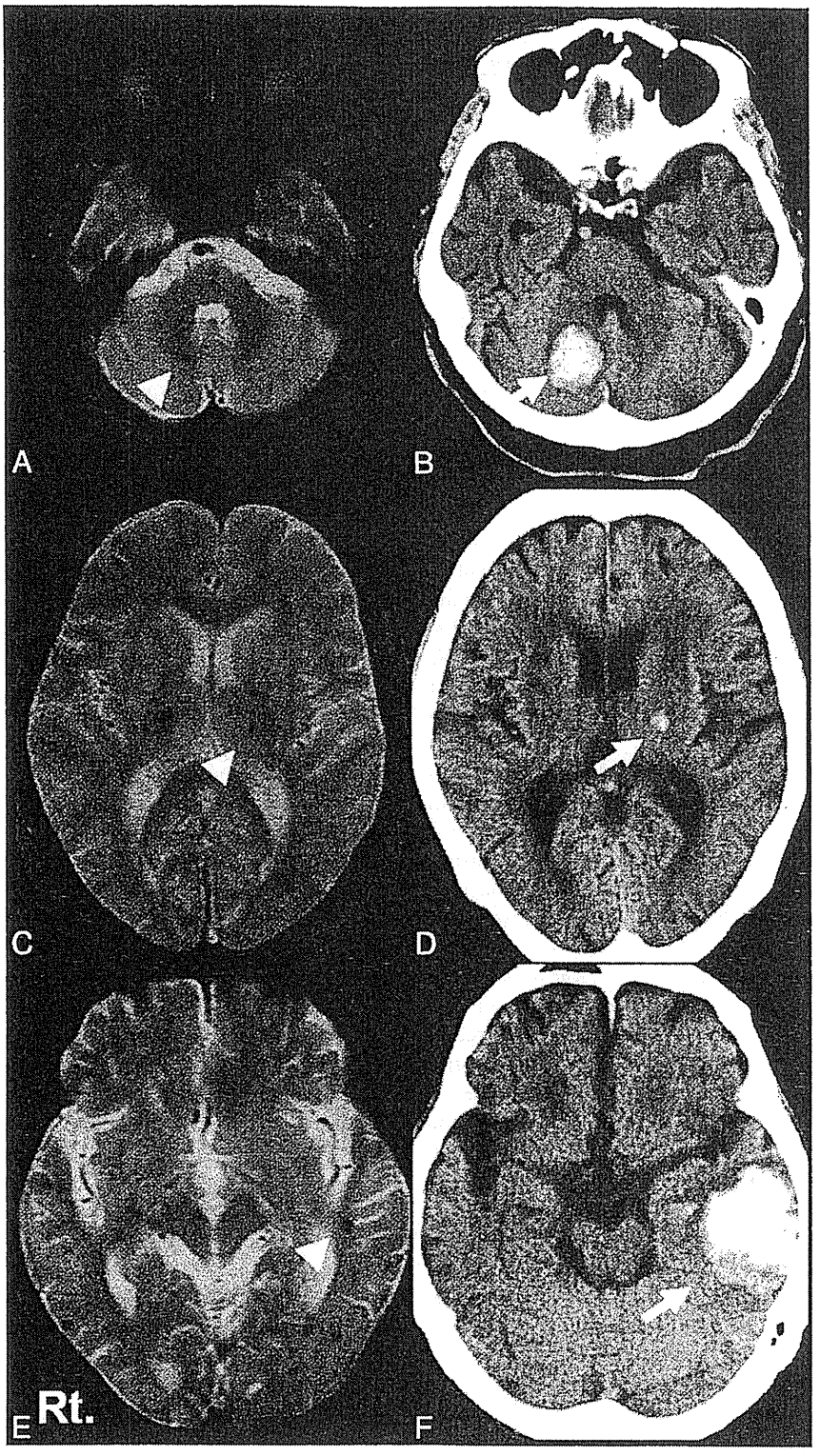

Fig 1. Representative cases. T2*-weighted MR image (*A*) and CT scan (*B*) in an 84-year-old patient. Recurrent right cerebellar hemorrhage (*arrow*) corresponds to the location of MBs detected 9 months before (*arrowhead*). T2*-weighted MR image (*C*) and CT scan (*D*) in an 80-year-old patient. Recurrent left thalamic hemorrhage (*arrow*) corresponds to the location of MBs detected 35 months before (*arrowhead*). T2*-weighted MR image (*E*) and CT scan (*F*) in an 85-year-old patient. Recurrent left lobar hemorrhage (*arrow*) corresponds to the location of MBs detected 3 months before (*arrowhead*).

$P = .020$). Other demographic and clinical data are shown in Table 1.

Positional Relationship between Recurrent ICH/LI and Previously Detected MBs. We evaluated the positional relationship between recurrent ICH/LI and previously detected MBs. In the recurrent ICH group, hematoma corresponded to the prior MBs in 21 of 34 patients (61.8%). Representative cases are shown in Fig 1. In contrast, LI corresponded to the

Table 2: Characteristics of corresponding and noncorresponding groups in patients with ICH

Characteristic	Corresponding (n = 21)	Noncorresponding (n = 13)	P Value[a]
Demographic data			
Median age, (yr) (range)	70 (51–84)	62 (55–78)	.188
Male sex, No. (%)	17 (81.0)	8 (61.5)	.151
Vascular risk factors			
Hypertension (%)	100	92.3	.934
Diabetes mellitus (%)	25.0	30.8	.681
Hypercholesterolemia (%)	26.3	61.5	.071
Antithrombotic therapy (%)	42.1	76.9	.075
Prior stroke subtype, No. (%)			
ICH	8 (38.1)	5 (35.7)	.886
LI	9 (42.9)	3 (21.4)	.282
ATBI	1 (4.8)	3 (21.4)	.279
CE	3 (14.3)	2 (14.3)	1.000
Hemorrhage volume, median (range) (cm^3)	15.1 (0.36–162)	3.43 (0.16–58.4)	.077
No. of MBs, median (range)	16 (4–73)	4 (1–49)	.014
Time from prior stroke, median day (range)	263 (58–1873)	150 (14–1407)	.748

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used

prior MBs in only 1 of 21 patients (4.8%) in the recurrent LI group. The correspondence ratio was, therefore, higher in the recurrent ICH group than in the recurrent LI group (OR, 32.3; 95% CI, 3.86–270.3; $P < .001$). The number of MBs and the time from prior stroke to the recurrent stroke were equivalent between the recurrent ICH group and the recurrent LI group (Table 1).

Among the ICH group, the number of MBs was higher in the "corresponding" group (median, 16; range, 4–73) than in the "noncorresponding" group (median, 4; range, 1–49; P = .001). The hemorrhage volume and the time from prior stroke were equivalent between both groups. Vascular risk factors, antithrombotic therapy, and prior stroke subtype were also equivalent between both groups (Table 2).

We also evaluated the association between the initial stroke subtype and correspondence between MB and stroke in the patients with recurrent ICH. Of the 34 patients in the recurrent ICH group, 13 patients had prior ICH and 21 had prior ischemic stroke. Among them, hematoma corresponded to the prior MBs in 8 of 13 patients with prior ICH (61.5%) and 13 of 21 patients with prior ischemic stroke (61.9%). The corresponding ratio was equivalent between the patients with prior ICH and the patients with prior ischemic stroke (P = .98).

Positional Relationship between Recurrent ICH and Previously Detected MBs in the Deep ICH Group versus the Lobar ICH Group. We evaluated the positional relationship between recurrent ICH and previously detected MBs for each type of hematoma (deep ICH versus lobar ICH). In the deep ICH group, hematoma corresponded to the prior MBs in 19 of 24 cases (79.2%) including 10 of 11 cases (90.0%) of thalamic hemorrhage, 6 of 7 cases (85.7%) of putaminal hemorrhage, 2 of 4 cases (50.0%) of cerebellar hemorrhage, and 1 of 2 cases (50.0%) of pontine hemorrhage (Fig 2). In contrast, in the lobar ICH group, hematoma corresponded to the prior MBs in only 2 of 10 patients (20.0%) (Fig 2). Among the patients with ICH, the correspondence ratio was higher in the deep ICH group than in the lobar ICH group (OR, 15.2; 95% CI, 2.42–95.3; $P < .002$). The hemorrhage volume, number of MBs, and the time from prior stroke to the recurrent ICH were equivalent between both groups (Table 3).

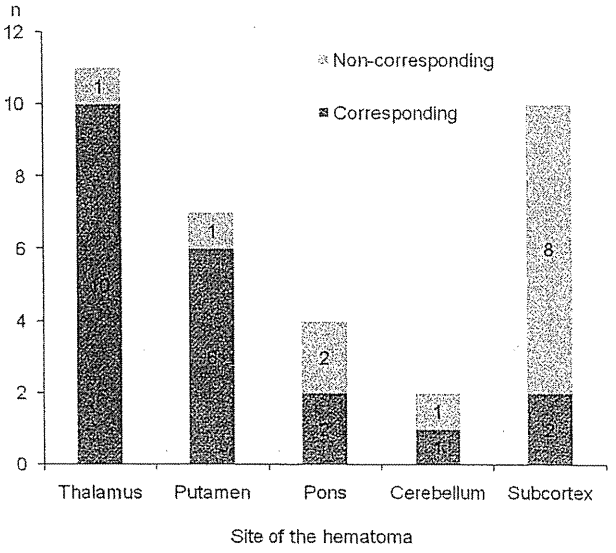

Fig 2. Correspondence of MBs in each part of the hematoma. The correspondence ratio was higher in the deep ICH group, particularly in thalamic and putaminal hemorrhage, than in the lobar ICH group.

Among the deep ICH group, the number of MBs in the whole brain and in the gray matter (thalamus, putamen, and caudate nucleus) was higher in the corresponding group (median, 16; range, 4–56; and median, 8; range, 3–28) than in the noncorresponding group (median, 4; range, 1–11; and median, 2; range, 1–8; P = .003 and P = .015). The time from prior stroke was equivalent between both groups. Vascular risk factors and prior stroke subtype were also equivalent between both groups. The rate of antithrombotic therapy was significantly higher in the noncorresponding group than in the corresponding group (Table 4).

Discussion

We found that the correspondence ratio was higher in patients with recurrent ICH than in patients with recurrent LI. In addition, among the patients with recurrent ICH, the correspondence ratio was higher in the deep ICH group, particularly in hemorrhage involving the putamen and thalamus, compared with the lobar ICH group.

Table 3: Characteristics of deep ICH and lobar ICH groups

Characteristic	Deep ICH (n = 24)	Lobar ICH (n = 10)	P Value[a]
Demographic data			
Median age (yr) (range)	69.5 (51–84)	66 (55–84)	.694
Male sex, No. (%)	17 (70.8)	8 (80.0)	.692
Vascular risk factors			
Hypertension (%)	100	90.0	.303
Diabetes mellitus (%)	26.1	20.0	1.000
Hyperlipidemia (%)	45.5	44.4	1.000
Antithrombotic therapy (%)	50.0	77.8	.237
Prior stroke subtype, No. (%)			
ICH	9 (37.5)	4 (40.0)	1.000
LI	11 (45.8)	1 (10.0)	061
ATBI	1 (4.2)	3 (30.0)	.067
CE	3 (12.5)	2 (20.0)	.618
Hemorrhage volume, median (range) (cm^3)	6.92 (0.16–66.9)	30.3 (0.69–162)	.287
No. of MBs, median (range)	13 (1–56)	8 (1–73)	.304
Time from prior stroke, median day (range)	292.5 (14–1873)	187.5 (79–1033)	.696
Correspondence to MBs, No. (%)	19 (79.2)	2 (20.0)	.002

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

Table 4: Characteristics of the corresponding and noncorresponding groups in the deep ICH group

Characteristic	Corresponding (n = 19)	Noncorresponding (n = 5)	P Value[a]
Demographic data			
Median age, (yr) (range)	70 (51–84)	67 (60–78)	.996
Male sex, No. (%)	15 (78.9)	2 (40.0)	.126
Vascular risk factors			
Hypertension (%)	100	100	-
Diabetes mellitus (%)	16.7	60.0	.078
Hypercholesterolemia (%)	29.4	60.0	.309
Antithrombotic therapy (%)	35.3	100	.035
Prior stroke subtype, No. (%)			
ICH	7 (36.8)	2 (40.0)	1.000
LI	9 (47.4)	2 (40.0)	1 000
ATBI	0 (0)	1 (20.0)	.208
CE	3 (15.8)	0 (0)	1.000
No. of MBs, median (range)			
In the whole brain	16 (4–56)	4 (1–11)	<.001
In the deep gray matter	8 (3–28)	2 (1–8)	.015
Time from prior stroke, median day (range)	322 (58–1873)	99 (14–1407)	.746

[a] χ^2 test, Fisher exact test, Student t test, or Mann-Whitney U test were used.

Only a few case reports[12,13] and several cases described in prospective studies performed other purposes[7,14] found that the subsequent ICH occurred in the same lesion in which prior MBs were detected. The present study is the first report focusing on the positional relationship between the subsequent ICH and the prior detected MBs in a relatively large number of patients.

Pathologically, MBs represent hemosiderin deposits that result from the fragility of small vessels in conditions such as lipohyalinosis, CAA, or arteriosclerosis.[1,2] The presence of MBs is closely associated with small-vessel diseases such as ICH and LI,[5,6] and it has been reported to be an important risk factor for subsequent stroke, particularly hemorrhagic stroke.[7-9]

The difference in correspondence ratios between ICH and LI may result from the difference in topology among ICH, LI, and MBs. Previous studies showed that MBs tend to be frequently present at the site of hypertensive ICH.[17,18] In contrast, MBs are seldom detected in the posterior limb of the internal capsule or the corona radiata,[18] which are the frequent sites of LI. This topographic difference may explain the discrepancy of the correspondence ratios between ICH and LI. However, it remains unclear why MBs are seldom detected in the frequent sites of LI and, furthermore, why the locations of prior MBs and recurrent LI do not coincide in other brain regions, even though both MBs and LI are based on microangiopathy. The close topologic association between prior MBs and recurrent ICH but not recurrent LI indicates that MBs are a form of small-vessel disease that is bleeding-prone.

The present study also reveals that the correspondence ratio in the deep ICH group was higher than that in the lobar ICH group, though the hemorrhage volume and the number of MBs were equivalent between both groups. Our findings may support the results of the Rotterdam Scan Study that MBs in a deep or infratentorial location were associated with hypertensive or atherosclerotic microangiopathy, whereas lobar MBs were related to CAA.[19] In the deep ICH group, close topologic association of prior MBs with subsequent ICH, particularly in the putamen and thalamus, suggests that subsequent hemorrhage may result from rerupture of microangiopathic vessels, such as those with lipohyalinosis in the deep brain area, which had been detected as MBs. In addition, the

higher number of MBs in the deep gray matter in the corresponding group suggests that MBs in this area may be a marker of the ongoing hypertensive microangiopathy and at risk for further subsequent ICH.

In contrast, the present study reveals the lower corresponding ratio between the prior MBs and subsequent ICH in the lobar ICH group. A recent pathologic study in patients with CAA suggested that the patients with many MBs demonstrated thicker amyloid-positive vessels than those with few MBs; therefore, CAA-related hemorrhage and MBs are based on different pathologies.[20] The CAA-related hemorrhage may result from rupture of amyloid-positive vessels, which are different from the vessels detected as MBs; the pathologic difference between ICH and MBs in CAA may result in the lower corresponding ratio in the lobar ICH group in the present study. However, we could not determine exactly whether the lobar hemorrhage resulted from CAA or hypertension because no patients enrolled in the present study were examined pathologically. This point is 1 of the limitations of the present study.

The other limitations should be noted. ICHs are often sizeable (particularly compared with LIs) and might, therefore, appear to coincide with a prior MBs simply because they cover a large volume of brain. It is even possible that deep ICHs, by occurring in a more confined anatomic territory than lobar ICHs, might be predisposed to coincide with prior MBs in the same territory. On the other hand, there was no difference in the hemorrhage volume between the corresponding group and the noncorresponding group overall in patients with ICH and between the deep ICH group and the lobar ICH group. Therefore, the effects of the hemorrhage volume for the corresponding ratio between the location of subsequent ICH and that of previously detected MBs may be excluded in the patients with ICH. In addition, although the corresponding ratio for patients with putamen/thalamic hemorrhages appeared to be higher than that in patients with pontine/cerebellar hemorrhages, this could be an aberration due to the small number of patients with pontine/cerebellar hemorrhage in our study. To clear up these limitations and confirm our results, we should perform prospective studies with a larger group of patients.

Conclusions

The close association between recurrent ICH and the location of previously detected MBs, especially in the putamen or thalamus, suggests that MBs represent hemorrhage-prone microangiopathy. In addition, the topologic distribution of MBs may be meaningful imaging information because the risk of subsequent ICH occurs in the same lesion in which MBs were previously detected. However, it still remains unclear whether the subsequent ICH in the location of previously detected MBs could be prevented with strict hypertension treatment or careful antithrombotic therapy, and prospective studies are needed to clarify these points.

Acknowledgments

We thank Naohisa Hosomi, MD, and Kayoko Ishihara, MD, for their advice in the drafting the manuscript and the radiologic technicians of our hospital for their acquisition of imaging data.

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BMC Neuroscience

RESEARCH ARTICLE Open Access

The pathophysiology of prospective memory failure after diffuse axonal injury - Lesion-symptom analysis using diffusion tensor imaging

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Abstract

Background: Prospective memory (PM) is one of the most important cognitive domains in everyday life. The neuronal basis of PM has been examined by a large number of neuroimaging and neuropsychological studies, and it has been suggested that several cerebral domains contribute to PM. For these activation studies, a constellation of experimental PM trials was developed and adopted to healthy subjects. In the present study, we used a widely used clinical PM assessment battery to determine the lesions attributable to PM failure, with the hypothesis that lesion-symptom analysis using diffusion tensor imaging (DTI) in subjects with diffuse axonal injury (DAI) can reveal the neuronal basis of PM in everyday life.

Results: Fourteen DAI patients (age: range of 18-36, median 24) participated in this study. PM failure was scored in the range of 0-6 using three sub-tests of the Rivermead Behavioural Memory Test. The PM scores of DAI patients were in the range of 2-6 (median 4.5, inter-quartile range 2.25). The severity of axonal injury following DAI was examined using fractional anisotropy (FA), one of the DTI parameters, at voxel level in each subject. We then obtained clusters correlated with PM failure by conducting voxel-based regression analysis between FA values and PM scores. Three clusters exhibited significant positive correlation with PM score, the left parahippocampal gyrus, left inferior parietal lobe, and left anterior cingulate.

Conclusions: This is the first lesion-symptom study to reveal the neuronal basis of PM using DTI on subjects with DAI. Our findings suggest that the neuronal basis of PM is in the left parahippocampal gyrus, left inferior parietal lobe, and/or left anterior cingulate. These findings are similar to those of previous activation studies with loading experimental PM tasks.

Background

Prospective memory (PM) is one of the most important cognitive domains in daily life. PM involves remembering to carry out intended actions at appropriate points in the future [1]. Even minimal reflection prompts the realization that the texture of our daily existence is inextricably bound to PM tasks. These tasks include mundane demands such as remembering to pick up bread on one's way home, remembering to mail the letter in one's briefcase, remembering to give one's housemate the message that a friend called, and remembering to load one's bicycle into the car for a ride after work. Theoretically, PM is considered a complex process involving at least four phases. The first phase is planning and encoding intention. The second phase is keeping the intention in mind and monitoring the environment to detect prospective cues while working on other tasks. The third phase is recognition of such cues, while recalling the intention and the intended action. Finally, the fourth is interruption of ongoing activity and then execution of the intended action [1-3]. PM thus appears to be a complex cognitive process depending on both the frontal system and hippocampal system [4]. This theory is supported by the findings of several neuropsychological studies [1-4] and a number of

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neuroimaging and neurophysiological studies performed to clarify the neuronal basis of PM [5-10]. In these activation studies, experimental PM trials were carefully designed to be adequate psychologically and suitable for PM in real-world situations [3].

Diffuse axonal injury (DAI) is one of the mechanisms of traumatic brain injury (TBI). DAI comprises primary microscopic injury of axons caused by acceleration-deceleration injury force, the pattern of which is more accurately described as multifocal, appearing throughout the deep and subcortical white matter and particularly common in midline structures including the splenium of the corpus callosum and brainstem on post-mortem pathological examination [11]. Cognitive sequelae of DAI are frequently observed, as is PM failure, which leads to problems in everyday life [12-17]. The pathophysiology of the cognitive sequelae of DAI is believed to stem from multiple injury of intracerebral connections. Diffusion tensor magnetic resonance imaging (DTI) has recently emerged as a valuable additional technique for evaluation of survivors with DAI. By measuring the degree of water diffusion anisotropy, quantitative information on the integrity of axons can be obtained at voxel level in the whole brain. It is reported that fractional anisotropy (FA), which is the best rotationally invariant scalar metric for measuring diffusion anisotropy, is broadly reduced in the white matter after DAI [18-20]. Furthermore, DTI parameters are now established as potential quantitative biomarkers for evaluation of the severity of axonal injury and prediction of functional abilities after DAI [19-24].

The purpose of this study was to clarify the neuronal basis of PM. We hypothesised that the pathological lesions contributing to PM failure could be mapped in patients with DAI by performing voxel-based regression analysis between FA values and PM scores. In this study, PM failure was measured by a clinical PM assessment battery, enabling us to determine the neuronal basis of PM in everyday life.

Results

Neuropsychological assessments

Basic patient characteristics and cognitive measurements are shown in Table 1. The average verbal intelligence quotient (VIQ) was 80.8 (standard deviation (SD) 18.2), performance intelligence quotient (PIQ) was 79.1 (SD 20.2), and full-scale intelligence quotient (FSIQ) was 78.5 (SD 20.3). The RBMT standardized profile scores were in the range of 7-24 (median 18, inter-quartile range 13.5). The PM scores were in the range of 2-6 (median 4.5, inter-quartile range 2.25). No correlation was observed between PM score and duration from onset (p = 0.39) or Glasgow Coma Scale (GCS) at the time of injury (p = 0.09). Although a correlation was observed between PM score and years of education (p = 0.037), there was no correlation between PM score and VIQ (p = 0.19), PIQ (p = 0.081), or FSIQ (p = 0.12).

Voxel-based regression analysis with PM score

Clusters with significant positive correlation with PM score were mapped in the right inferior parietal lobes (peak T value = 9.26, peak Z score = 4.93, peak Montreal Neurological Institute (MNI) coordinates x = 56, y = -32, z = 26; nearest Brodmann's area (BA) 40), left parahippocampal gyrus (peak T value = 6.96, peak Z score = 4.33, peak MNI coordinates x = -20, y = -60, z = -8, nearest BA 19), left inferior parietal lobe (peak T value = 6.67, peak Z score = 4.23, peak MNI coordinates x = -30, y = -34, z = 38, nearest BA 40), and left anterior cingulate gyrus (peak T value = 6.34, peak Z score = 4.12, peak MNI coordinates x = -4, y = 38, z = 12, nearest BA 32) (Figure 1 and Table 2).

Visual checking of extracted clusters revealed that the cluster located in the right inferior parietal lobe was in the Sylvian fissure, which resulted in lower FA values in subjects whose PM scores were lower. It appeared that reduction of FA in this region did not reflect axonal injury, and instead cortical atrophy. We therefore excluded the cluster located in the right inferior parietal lobe from the group of significant clusters.

The other three clusters located in the left parahippocampal gyrus, left anterior cingulate, and left inferior parietal lobe consisted mainly of subcortical white matter with partial involvement of cerebral cortex. The correlation coefficients between FA value and PM score in each of these clusters was following; left parahippocampal gyrus (r = 0.89, p < 0.001), left inferior parietal lobe (r = 0.93, p < 0.001), and left anterior cingulate (r = 0.90, p < 0.001), respectively. Moreover, there was no significant difference in FA value between DAI subjects with full PM score (score = 6) and normal volunteers in these clusters [left parahippocampal gyrus (p = 0.20), left inferior parietal lobe (p = 0.79), and left anterior cingulate (p = 0.29)] (Figure 2). There were no significant correlation between FA values and times from the injury in each cluster [left parahippocampal gyrus (r = 0.33, p = 0.26), left inferior parietal lobe (r = 0.17, p = 0.56), and left anterior cingulate (r = 0.18, p = 0.54)].

Discussion

Multiple axonal lesions of varying severity are scattered throughout the white matter in the brains of DAI patients, and various cognitive sequelae are observed depending on the severity and location of such lesions. Since PM function depends on intact intra-cerebral networks in several cognitive domains, disruption of these connections results in the failure of PM. DTI is a suitable method for detecting such disconnections, and is

Table 1 Clinical characteristics and results of cognitive measures in patients with DAI

Age	Gender	Education (years)	Duration (months)	GCS	WAIS-III			RBMT	
					VIQ	PIQ	FSIQ	SPS	PM
24	M	16	49	4	75	78	74	14	5
36	M	12	9	3	60	60	57	17	4
27	M	16	15	7	85	74	78	16	4
21	M	12	51	7	77	86	79	22	6
20	M	11	13	3	84	94	87	7	4
19	M	9	6	3	91	97	93	20	4
22	F	12	27	3	n.a.	n.a.	n.a.	8	2
22	M	16	24	7	115	117	118	23	6
34	M	12	3	3	65	60	60	8	3
27	M	16	5	3	111	106	109	22	6
24	M	16	5	3	94	75	88	19	5
18	M	12	3	3	60	47	50	9	2
33	M	14	6	6	63	63	60	23	6
36	M	16	12	3	70	71	68	24	6
[b]24		[a]13.6 (2.4)	[a]16.3 (16.1)	[b]3 (2)	[a]80.8 (18.2)	[a]79.1 (20.2)	[a]78.5 (20.3)	[b]18 (13.5)	[b]4.5 (2.25)

F: female, M: male, GCS: Glasgow Coma Scale, WAIS-III: Wechsler Adult Intelligence Scale-III, VIQ: verbal intelligence quotient, PIQ: performance intelligence quotient, FSIQ: full-scale intelligence quotient, RBMT: Rivermead Behavioural Memory Test, SPS: standardized profile score, PM: prospective memory, n.a.: not applicable. [a]: mean (standard deviation), [b]: median (inter-quartile range)

therefore considered useful for identifying certain domains associated with PM. We believe it is possible to determine the brain region attributable to each cognitive function affected in these patients by comparing cognitive parameters and FA values, which can be used to evaluate the severity of axonal injury at voxel level. With this hypothesis, we sought to locate lesions attributable to PM failure after DAI. To the best of our knowledge, no previous study has mapped the lesions attributable to PM failure through voxel-based lesion-symptom analysis using DTI. In this study, three clusters significantly correlated with PM failure were found in the left parahippocampal gyrus, left inferior parietal lobe, and left anterior cingulate.

Of the clusters correlated with PM failure, that in the left parahippocampal gyrus is consistent with the findings of previous neuroimaging studies with loading of experimental PM tasks. In previous PET studies, activation of the left parahippocampal gyrus was observed during loading of experimental PM tasks, and activation of this region was thought to play a role in recognition of cues triggering the performance of intended actions [6,7,10,25]. The second cluster correlated with PM failure was detected in the left inferior parietal lobe. Voxel-based regression analysis with Trail Making Test-B, which was conducted in the same DAI subjects, showed that the cluster correlated with Trail Making Test-B was in the left inferior parietal lobe, in a location close to the cluster correlated with PM (Additional file 1and 2). In functional neuroimaging studies, cortical activation in this area was observed on loading of the Wisconsin Card Sorting Test and Trail Making Test, suggesting that neuronal activity in this region reflects the cognitive process of set shifting [26,27]. Set shifting is believed to participate in PM, e. g. in monitoring of the environment for cues to re-instantiate an intention while performing other tasks (i.e. the second phase of the PM process) or inhibiting other activities at the critical time for performance of the intended action (i.e. the fourth phase of the PM process). It also appeared that lesions in the left anterior cingulate worsen PM function. This finding is consistent with a previous lesion-symptom analysis study using computed tomography. In that study, lesions in the left anterior cingulate causing PM failure were associated with failure of recall of the intention and intended action [2]. These findings suggested that PM failure after DAI might reflect operation of the supporting retrospective components of PM, as well as those related more specifically to maintenance of an intention.

Previous studies using functional neuroimaging and event-related potential recording have revealed a relationship between the right inferior parietal lobe and PM function [1,7-10,28]. In the present study, a correlation between FA value and PM score was found for the cluster in the right inferior parietal lobe. However, post-hoc testing revealed that lower FA values in this cluster could be assigned to the cluster beyond the Sylvian fissure. Further study using accurate voxel-based morphometry is needed to determine whether atrophy of this region contributes to PM failure in patients with DAI.

The localization of the clusters correlated with PM failure was strikingly similar to that of regions activated during experimental PM tasks in previous activation