Stroke

CLINICAL AND POPULATION SCIENCES

Relation Between the Corticospinal Tract State and Activities of Daily Living in Patients With Intracerebral Hemorrhage

Sung Ho Jang, MD; Eun Bi Choi, PT, MS

BACKGROUND AND PURPOSE: We investigated the relation between the ipsilesional corticospinal tract (CST) state and activity of daily living independence in patients with chronic intracerebral hemorrhage.

METHODS: Fifty-six consecutive patients with unilateral intracerebral hemorrhage and 38 healthy control subjects were recruited for this study. The Motricity index and the modified Barthel index were used to evaluate motor function of the affected extremities and activity of daily living independence, respectively. The diffusion tensor imaging parameter values for fractional anisotropy (FA) and voxel number (VN) of the CST were determined. Ratios of the ipsilesional to the contralesional CST measures were calculated and are presented as the CST-ratio (FA value and VN).

RESULTS: The FA value and VN of the ipsilesional CST and the CST-ratio in the patient group were lower than those of the control group ($P<0.05$). There was a strong positive correlation between the Motricity index score of the affected extremities and the modified Barthel index score ($P<0.05$), while the FA value and VN of the ipsilesional CST and the CST-ratio showed moderate and strong positive correlations with the Motricity index and modified Barthel index scores, respectively ($P<0.05$). In addition, the VN of the ipsilesional CST showed excellent utility as a classifier, whereas the FA value of the ipsilesional CST and the FA value and VN of the CST-ratio showed good classifier utility ($P<0.05$).

CONCLUSIONS: We demonstrated that impairment of activity of daily living independency was closely related to the injury severity of the ipsilesional CST in patients with chronic intracerebral hemorrhage. In addition, the injury severity of the ipsilesional CST can be used to classify the degree of activity of daily living independency.

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Key Words: activities of daily living ■ cerebral hemorrhage ■ diffusion tensor imaging ■ pyramidal tract

Activity of daily living (ADL) reflects the ability to perform basic tasks of living independently, such as self-care and functional mobility, and is known to be impaired in ≈25% to 74% of patients with stroke.[1,2] Impairment of ADL independence can result in patients having decreased quality of life and social participation.[3] ADL independence is affected by neurological impairments, including motor weakness, sensory loss, language dysfunction, and impaired consciousness.[3,4] Among the neurological impairments, motor weakness can be mainly caused by the corticospinal tract (CST) injury, the most important neural tract for motor function in the human brain.[5] Many studies have demonstrated differences in motor function according to the state of the ipsilesional CST in patients with stroke.[5–7] Consequently, ADL independence can differ according to the ipsilesional CST

Correspondence to: Eun Bi Choi, PT, MS, Department of Physical Medicine and Rehabilitation, College of Medicine, Yeungnam University 317-1, Daemyungdong, Namku, Daegu 705-717, Republic of Korea. Email ceb0808@hanmail.net

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Nonstandard Abbreviations and Acronyms

ADL	activity of daily living
AUC	area under the curve
CST	corticospinal tract
DTI	diffusion tensor imaging
DTT	diffusion tensor tractography
FA	fractional anisotropy
ICH	intracerebral hemorrhage
MBI	modified Barthel index
MI	Motricity index
ROC	receiver operating characteristic
VN	voxel number

state. A few studies have reported that impairment of ADL independence in the acute or subacute stages of stroke is related to the ipsilesional CST state.[8–10] However, precise estimation of the ipsilesional CST state during these stages can be difficult due to the presence of perilesional edema or the Wallerian degeneration stage.[11–16] In addition, during these stroke stages, a precise examination of motor weakness can be difficult due to cognitive dysfunction and several kinds of apraxia or visuospatial dysfunction.[13–15] As a result, assessment of the relation between the ipsilesional CST state and ADL independence in patients with stroke would be more accurate in the chronic stage than in the acute or subacute stages. In addition, knowledge about the relation between the ipsilesional CST state and ADL independence could be useful in setting rehabilitation strategy aimed at ADL independence during the recovery phase in patients with stroke with motor weakness.

The development of diffusion tensor tractography (DTT), an imaging approach derived from diffusion tensor imaging (DTI), has enabled 3-dimensional reconstruction and estimation of the CST.[17,18] The CST state can be determined by evaluating DTT parameters, including configuration, the presence of discontinuation, fractional anisotropy (FA) value, and voxel number (VN).[19,20] The FA value indicates the state of white matter organization by indicating the degree of directionality and integrity of white matter microstructures; in contrast, the VN indicates the number of voxels included in a neural tract, which is deemed representative of the total number of fibers within the tract.[19,20] Since the introduction of DTI, impairment of ADL independence was reported to be closely related to specific brain regions, including the CST, based on FA mapping in patients in the chronic stage of stroke (infarct or hemorrhage).[21] However, no study has reported a relation between the ipsilesional CST state and ADL independence based on analysis of DTT data for the entire CST of patients with chronic stroke. For the present study, we hypothesized that impairment of ADL independence could be related to the ipsilesional CST state in chronic patients with intracerebral hemorrhage (ICH). Thus, we used DTT to investigate the relation between the ipsilesional CST state and ADL independence in chronic patients with ICH.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Subjects

Fifty-six consecutive patients (38 male, 18 female; mean age, 50.63±10.92 years, range, 24–69 years) who visited the rehabilitation department of a university hospital and 38 age- and sex-matched healthy control subjects without previous history of psychiatric or neurological disease (23 male, 15 female; mean age, 46.68±8.90; range, 33–65 years) were recruited for this study to compare the CST state. Patients were recruited consecutively according to the following inclusion criteria (Figure I in the Data Supplement): (1) first-ever stroke; (2) spontaneous unilateral supratentorial ICH that was confirmed by a neuroradiologist; (3) age at onset of ICH: 20 to 69 years; (4) DTI scans obtained at the chronic stage (>3 months after stroke onset); (5) no previous history of neurological or psychiatric disease. Patients with cognitive problems (Mini-Mental State Examination score of <24) were excluded. This study was conducted cross-sectionally and reported in accordance with STROBE guidelines (STROBE checklist in the Data Supplement). The study protocol was approved by the institutional review board of Yeungnam university hospital (approval No. YUMC-2021-03-014). Control subjects provided written informed consent.

Clinical Evaluation

Motor function and ADL independence were evaluated by physiatrists (Motricity index [MI]) and occupational therapist (modified Barthel index [MBI]) who were blinded to DTI data at the time of DTI scanning using MI for motor function of the affected extremities and the MBI for impairment of ADL independence.[22,23] The MI score (maximum score: 100) is a modification of the Medical Research Council scoring system.[22] The total MI score is the average of upper (shoulder flexor, elbow flexor, and prehension) and lower (hip flexor, knee extensor, and ankle dorsiflexor) MI scores, and each joint of extremities is scored as follows: the MI score except for prehension: 0 (no contraction), 28 (palpable contraction, but no visible movement), 42 (movement without gravity), 56 (movement against gravity), 74 (movement against a resistance lower than the resistance overcome by the healthy side), 100 (movement against a resistance equal to the maximum resistance overcome by the healthy side); the MI score for prehension: 0 (no movement), 33 (beginning of prehension), 56 (prehension of the object without gravity), 65 (prehension of the object against gravity), 77 (prehension against slight manual resistance applied to the object), 100 (prehension against resistance identical of the resistance overcome by the healthy hand).[22] The MBI (maximum score: 100) was used to evaluate ADL independence by assessing 10 items including the patient's personal hygiene, bathing, feeding, toileting, stair up and down, dressing, defecation, voiding, ambulation, and bed transfer.[23] Each 10 item is scored 5 different weights of rating scales: unable, attempts but unsafe, moderate help, minimal help, and fully independent.[23] A higher MBI score represents a higher independence in performing ADL.[23] The MBI cutoff score was 75 (≥75 for good outcome and ˂75 for poor outcome).[24] The reliability and validity of the MI and MBI have been well established.[22,23]

Diffusion Tensor Imaging

DTI scanning was performed at 16.08±18.89 months after the onset of ICH using a 6-channel head coil on a 1.5T Philips Gyroscan Intera scanner (Hoffman-LaRoche, Best, Netherlands) by performing single-shot, spin-echo planar imaging. For each of the 32 noncollinear diffusion sensitizing gradients, 67 contiguous slices were acquired parallel to the anterior commissure-posterior commissure line. Imaging parameters were as follows: acquisition matrix=96×96, reconstructed to matrix=192×192, field of view=240 mm×240 mm, repetition time=10 398 ms, echo time=72 ms, parallel imaging reduction factor (SENSE factor)=2, echo planar imaging factor=59, b=1000 s/mm^2, number of excitations=1, and slice thickness=2.5 mm. Eddy current correction was applied to correct for head motion effects and image distortion by using software included in the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB) Software Library (FSL: www.fmrib.ox.ac.uk/fsl).[25] Fiber tracking for CST evaluation was performed using DTI-Studio software (CMRM, Johns Hopkins Medical Institute, Baltimore, MD). For reconstruction of the CST, a seed region of interest was placed on the anterior blue portion of the mid-pons on the axial image of the color map, and a target region of interest was placed on the anterior blue portion of the low-pons on the axial image of the color map.[26,27] Fiber tracking was started when the seed voxel FA value was >0.2 and ended at the voxel with a fiber assignment value >0.2 and a tract turning-angle of <60°.[27] The FA value and VN of the CST were measured in both hemispheres. Ratios of the ipsilesional CST to the contralesional CST measures (FA value and VN) were calculated and presented as CST ratios. In addition, the integrity of the ipsilesional CST in the patient group was evaluated according to its DTT configuration status as either preserved integrity or discontinuous tract integrity at or below the lesion (Figure 1). Demographic data for the patient and control groups are summarized in Table 1.

Statistical Analysis

Statistical analysis was performed by using SPSS 21.0 for Windows (SPSS, Chicago, IL). The distribution of data including DTT parameters (FA value and VN) was not satisfied in the normality test using the Kolmogorov-Smirnov test (P<0.05). The chi-squared test (χ^2; sex) and Mann-Whitney U test (age) were used to examine the demographic data. Multivariate linear regression analysis was performed for determining the association of clinical data (MI and MBI scores) and DTT parameters (FA value and VN) adjusted for the demographic variables (age, sex, and duration to DTI since stroke) that could affect the correlation. We presented the regression coefficients, CI, and P for each variables.

Mann-Whitney U test was used to evaluate of differences in DTT parameters (FA value and VN) of the ipsilesional CST and in CST ratios between the patient and control groups, as well as to assess differences in clinical data (MI and MBI scores)

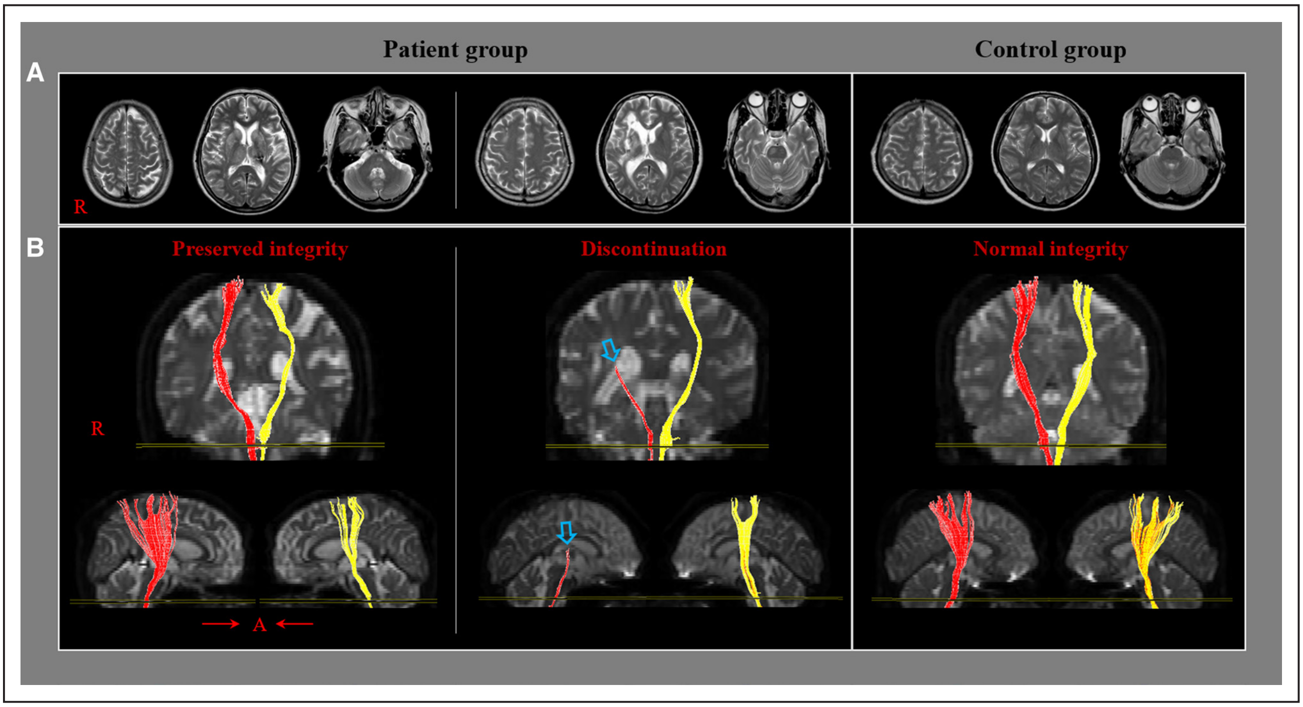

Figure 1. Brain images and diffusion tensor tractography results for representative subjects in the patient (preserved corticospinal tract integrity: 57-y-old female, discontinuation of corticospinal tract integrity: 48-y-old female) and control (51-y-old female) groups.

A, T2-weighted brain magnetic resonance images acquired at the time of diffusion tensor imaging scanning. **B**, Results of diffusion tensor tractography of the corticospinal tract: discontinuation in the ipsilesional corticospinal tract at the lesion (blue arrow).

Table 1. Demographic and Clinical Data of the Patient and Control Groups

Characteristic	Patient group (n=56)	Control group (n=38)
Age, y	50.63 (10.92)	46.68 (8.90)
Sex (male/female)	38/18	23/15
MMSE	27.34 (2.31)	…
Duration to DTI, mo	16.08 (18.89)	…
Lesion location (right/left)	29/27	…
MI	65.30 (23.24)	…
MBI	67.70 (19.06)	…

Values represent mean (±SD). DTI indicates diffusion tensor imaging; MBI, modified Barthel index; MI, motricity index; and MMSE, Mini-Mental State Examination.

according to the ipsilesional CST state in the patient group. Statistical significance was accepted for *P* of <0.05.

The Spearman correlation coefficient was used to determine the correlation between clinical data (MI and MBI scores) and DTT parameters (FA value and VN) of the ipsilesional CST and the CST-ratio. Significance of a detected relationship was accepted when the test's *P* was <0.05. A correlation coefficient was interpreted as strong when ≥0.60, as moderate when between 0.40 and 0.59, weak when between 0.20 and 0.39, and very weak when ≤0.19.[28]

Receiver operating characteristic (ROC) analysis was performed to determine the area under the curve (AUC), CI, sensitivity, specificity, and cutoff values of the DTT parameters. Statistical significance of the AUC was accepted for *P* of <0.05. The classification utility of the DTT parameters was categorized by assessing the AUC as follows: excellent, 0.90 to 1.00; good, 0.80 to 0.90; fair, 0.70 to 0.80; poor, 0.60 to 0.70; fail, 0.50 to 0.60.[29] The cutoff values of each DTT parameter for discriminating patients with a good outcome from those with a poor outcome were determined by calculating the Youden index (J=sensitivity+specificity−1).[30]

RESULTS

The results of multivariate linear regression analysis is summarized in Table I in the Data Supplement. The FA value and VN of the ipsilesional CST and CST-ratio were correlated with the MI and MBI scores, respectively ($P<0.05$). After adjusting for age, sex, and duration to DTI, significant correlations were observed between the FA value and VN of the ipsilesional CST and CST-ratio, and the MI and MBI scores ($P<0.05$). A summary of the comparison of DTT parameter and clinical (MI and MBI scores) data of the patient and control groups is presented in Table 2. Significant differences were observed in the FA value and VN of the ipsilesional CST between the patient and control groups ($P<0.05$). In terms of CST ratios, the FA value and VN in the patient group were significantly lower than those of the control group ($P<0.05$). Regarding DTT configuration, patients with preserved integrity (22 patients [39.3%]) of the ipsilesional CST had higher MI and MBI scores than those of patients with discontinuation (34 patients [60.7%]) of the ipsilesional CST.

Correlations between the clinical (MI and MBI scores) and DTT parameter (FA value and VN) results for the CST are summarized in Table 3. The MI score of the affected extremities showed strong positive correlations with the MBI score ($r=0.769$, $P<0.05$), the FA value and VN of the ipsilesional CST (FA value: $r=0.738$, $P<0.05$; VN: $r=0.814$, $P<0.05$), and the FA value and VN of the CST-ratio (FA value: $r=0.744$, $P<0.05$; VN: $r=0.758$, $P<0.05$; Figure II in the Data Supplement). In addition, the MBI score had strong positive correlations with the VN of the ipsilesional CST and the CST-ratio (VN of ipsilesional CST: $r=0.722$, $P<0.05$; VN of the CST-ratio: $r=0.666$, $P<0.05$) (Figure III in the Data Supplement). Moderate positive correlations were observed between the MBI score and the FA values of the ipsilesional CST and the CST-ratio (FA value of ipsilesional CST: $r=0.598$, $P<0.05$; FA value of CST-ratio: $r=0.561$, $P<0.05$).

Results of the ROC analysis of the DTT parameters of the CST are presented in Table 4. The AUC for the VN of the ipsilesional CST (AUC, 0.91 [95% CI, 0.83-0.98]) was the largest among the tested DTT parameters and

Table 2. Comparison of Clinical and DTT Parameter Data for the CST in the Patient and Control Groups

	Patient group		Control group	*P* value
Ipsilesional CST				
FA	0.42 (0.10)		0.56 (0.03)	<0.001*
VN	511.77 (564.22)		1674.07 (476.29)	<0.001*
CST-ratio				
FA	0.79 (0.19)		0.97 (0.04)	<0.001*
VN	0.36 (0.36)		0.96 (0.18)	<0.001*
DTT configuration (n [%])	Preserved integrity (22 [39.3])	Discontinuation (34 [60.7])	…	…
MI	84.94 (13.60)	52.59 (18.94)	…	<0.001*
MBI	81.77 (14.50)	58.59 (15.93)	…	<0.001*

Values represent mean (±SD). CST indicates corticospinal tract; DTT, diffusion tensor tractography; FA, fractional anisotropy; MBI: modified Barthel index; MI, motricity index; and VN, voxel number

*Significant difference ($P<0.05$).

Table 3. Correlations Between Diffusion Tensor Tractography Parameters for the CST and Patients' Clinical Data

			Ipsilesional CST		CST-ratio	
		MBI	FA	VN	FA	VN
MI	*r*	0.769	0.738	0.814	0.744	0.758
	P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
MBI	*r*	…	0.598	0.722	0.561	0.666
	P	…	<0.001*	<0.001*	<0.001*	<0.001*

CST indicates corticospinal tract; FA, fractional anisotropy; MBI, modified Barthel index; MI, motricity index; and VN, voxel number.
*Significant difference (P<0.05).

showed excellent utility as a classifier (P<0.05; Figure 2). By contrast, good utility was observed for the FA value of the ipsilesional CST (AUC, 0.83 [95% CI, 0.72–0.94]) and the FA value (AUC, 0.82 [95% CI, 0.71–0.93]) and VN (AUC, 0.89 [95% CI, 0.80–0.97]) of the CST-ratio (P<0.05). The cutoff levels for the FA value and VN of the ipsilesional CST for outcome classification were 0.43 and 307.50, respectively, with sensitivities of 0.91 and 0.95 and specificities of 0.74 and 0.80, respectively. The cutoff values for the FA value and VN of the CST-ratio were 0.82 and 0.18, respectively, with sensitivities of 0.86 and 0.95 and specificities of 0.74 and 0.71, respectively.

DISCUSSION

In this study, regarding the relation between the ipsilesional CST state and ADL in ICH, our results are summarized as follows. (1) The mean FA value and VN of the ipsilesional CST and the CST-ratio in the patient group were lower than those of the control group. (2) The MI and MBI scores were lower in patients with ipsilesional CST discontinuation than in patients with preserved integrity of the ipsilesional CST. (3) The MI score of the affected extremities revealed strong positive correlation with the MBI score. In addition, the FA values and VNs of the ipsilesional CST and the CST-ratio had moderate and strong positive correlations with the MBI score, respectively. (4) ROC curve analysis of the VN of the ipsilesional CST showed excellent classification utility, while the FA value of the ipsilesional CST, and the FA value and VN of the CST-ratio revealed good utility capacity.

Regarding DTT parameters, a decreased FA value or VN of a neural tract is indicative of an injury to that neural tract.[27,31] Therefore, decrements in FA value and VN of the ipsilesional CST in the patient group than the control group suggest the presence of neural injuries to the ipsilesional CST in the patient group. The FA value and VN of the CST-ratio reflect the degree of asymmetricity between the ipsi- and contralesional CSTs. Thus, decrements in the FA value and VN of the CST-ratio in the patient group compared with the control group indicate the presence of ipsilesional CST injury.

The result showing that the MI score has a strong positive correlation with the MBI score indicates that motor function of the affected extremities is an important factor affecting ADL. On the other hand, the MBI score showed a positive correlation with DTT parameters and their ratios (FA: moderate, VN: strong), indicating that both the directionality (FA) and amount of the remaining neural fibers (VN) of the ipsilesional CST affect the level of ADL independence. However, the results indicate that the amount of remaining neural fibers (VN) of the ipsilesional CST has a greater effect than the level of directionality (FA).

ROC analysis is a standard method for performance assessment, and the obtained ROC curve can be used to determine a cutoff value to classify a subject's outcome as either positive or negative.[29,30] The utility of a parameter as a classifier can be determined by assessing the parameter's AUC.[29] An AUC of 0.5, which indicates a random classification of patients with good or poor outcomes, indicates the nonpractical utility of a parameter as a classifier; in contrast, an AUC of 1.0 suggests the parameter is a perfect classifier.[29] In this study, the ROC-based analysis of the utility of DTT parameters (the ipsilesional CST: FA, good; VN, excellent; the CST-ratio: FA, good; VN, good) suggests that the directionality (FA) and amount of remaining neural fibers (VN) of the ipsilesional CST and the CST-ratio were useful as classifiers. However, the amount of remaining neural fibers (VN) of the ipsilesional CST had better utility than the directionality of fibers (FA) of the ipsilesional CST. In addition, the sensitivities (the proportion of subjects classified as positive among the actual positive subjects) of the VN of

Table 4. Receiver Operating Characteristic Curve Analysis of Diffusion Tensor Tractography Parameters of the Corticospinal Tract for Discriminating the Outcome of Activities of Daily Living Independency

	Cutoff value	AUC (95% CI)	Sensitivity	Specificity	*P* value
Ipsilesional CST					
FA	0.43	0.83 (0.72–0.94)	0.91	0.74	<0.001*
VN	307.50	0.91 (0.83–0.98)	0.95	0.80	<0.001*
CST-ratio					
FA	0.82	0.82 (0.71–0.93)	0.86	0.74	<0.001*
VN	0.18	0.89 (0.80–0.97)	0.95	0.71	<0.001*

AUC indicates area under the curve; CST, corticospinal tract; FA, fractional anisotropy; and VN, voxel number.
*Significant differences (P<0.05).

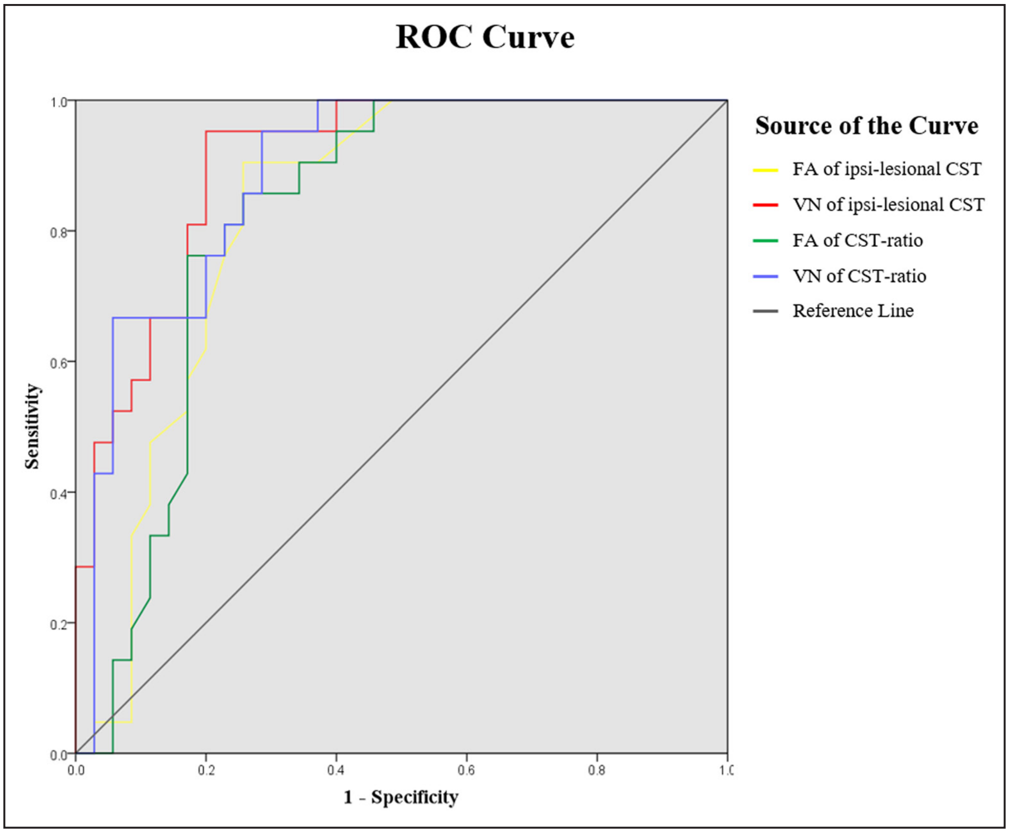

Figure 2. Receiver operating characteristics curves demonstrating the utility of discriminating patients with a good outcome from those with a poor outcome using diffusion tensor tractography parameters (fractional anisotropy [FA] and voxel number [VN] of the ipsilesional corticospinal tract [CST] and CST-ratio).
The area under the curve for the VN of the ipsilesional CST (red line) is the largest among the diffusion tensor tractography parameters (area under the curve, 0.91; $P<0.05$).

the ipsilesional CST and the CST-ratio were higher than the respective sensitivities of the FA values. The highest specificity (the proportion of those classified as negative among the actual negative subjects) was detected in the VN of the ipsilesional CST.[29] These results suggest that the level of remaining neural fibers of the ipsilesional CST had the highest sensitivity and specificity in classifying patient ADL outcome as good or poor. Based on our analysis, for a patient with unknown ADL independence and above the cutoff value in the VN of ipsilesional CST, there is a 95% possibility that the patient's ADL outcome would be correctly classified as positive. Similarly, when the patient's VN is below the cutoff value, there is an 80% possibility that the patient's ADL outcome would be correctly classified as negative. As a result, to predict a patient's ADL outcome as good or poor, both the directionality (FA) and the amount of neural fibers (VN) of the ipsilesional CST are useful as classifier utilities, with the VN of the ipsilesional CST showing the highest utility, sensitivity, and specificity. As a result, the remaining neural fibers of the ipsilesional CST appeared to be the most appropriate parameter for classifying a patient's ADL outcome as good or poor.

A few studies, by assessing DTI or DTT, have reported on the relation between the CST state and ADL independence in patients with stroke during the acute or subacute stages.[8–10,16] In 2010, Radlinska et al[8] used DTI to demonstrate that ADL independence within 3 weeks after onset was more impaired in ischemic patients with the CST involved compared with ischemic patients without CST involvement. In 2013, Vargas et al,[9] using DTT, reported that the FA and FA ratio of the ipsilesional CST were related with ADL independence in ischemic patients at 3 weeks after onset. In 2015, Imura et al[10] demonstrated a relation between ADL independence and FA value of the ipsilesional CST within 10 days after onset in patients with stroke by using DTT. In contrast, Kim et al[16] reported that ADL independence was not related with any DTI or DTT parameters of the ipsilesional CST within 2 months after onset in patients with stroke. However, precise estimation of motor weakness and the ipsilesional CST state during the acute or subacute stages can be limited because of various reasons, which are mentioned in the introduction.[11–16] Regarding the chronic stage of stroke, only one study was reported by Choi et al.[21] The authors demonstrated that the FA value of the corona radiata in the contralesional hemisphere, which contains the CST was associated with ADL independence in patients with stroke.[21] However, in this study, the entire CST was not estimated like our study because the FA value was measured 2-dimensionally in the corona radiate, which contains other neural tracts such as the corticoreticular tract and spinothalamic tract as well as the CST.[32,33] In addition, their results were different with our results. As a result, to the best of our knowledge, the present study is the first to demonstrate a relation between the ipsilesional CST state and ADL independence, which was estimated the entire CST using DTT in patients with chronic ICH. Our results appeared to be generally coincided with the results of above previous studies except for studies by Kim et al[16] and Choi et al.[21]

However, some limitations of this study should be considered. First, DTT analysis can overestimate or underestimate neural fiber status in areas with crossing fibers or fiber complexity. Second, because the patients who visited the rehabilitation department of a university hospital were recruited in this study, there is a possibility that the patients in our study may have had more severe clinical features than those in a more general population of patients with ICH. Therefore, further studies to overcome these limitations should be undertaken.

In conclusion, we have demonstrated that impairment of ADL independency is closely related to the severity of ipsilesional CST injury in patients with chronic ICH. In addition, the injury severity of the ipsilesional CST can be useful in discriminating the degree of ADL independency. Our results suggest that the state of the ipsilesional CST can be an important factor in ADL outcome in patients with chronic ICH. As a result, facilitation of recovery of an injured ipsilesional CST during the ICH recovery phase would be important for achieving ADL independence during the chronic stage. In addition, knowledge about the ipsilesional CST state would be useful in rehabilitation aimed at ADL independence in patients with motor weakness following ICH.

ARTICLE INFORMATION

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Affiliations

Department of Physical Medicine and Rehabilitation, College of Medicine, Yeungnam University, Republic of Korea.

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Disclosures

None.

Supplemental Material

STROBE checklist
Online Table I
Online Figures I–III

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