

Corpus Callosum Integrity Predicts Functional Outcomes in Acute Stroke: A Probabilistic Structural Connectivity Study

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Background and Purpose Stroke impairs cognition and movement. Although clinical severity and infarct volume can predict functional outcomes, variability in patient responses requires advanced structural and functional connectivity methods. Disconnection markers were tested to predict functional outcomes after acute ischemic stroke using diffusion tensor imaging.

Methods A probabilistic approach was used to quantify brain damage from white matter (WM) disconnections affecting cortical areas, using lesion masking on a tractography atlas and parcellation of gray matter into functional network nodes. Forty-three patients with acute ischemic stroke were grouped according to functional improvement (change in modified Rankin Scale score from 3–5 at discharge to 0–2 at 3-month follow-up). Significantly different structural disconnection measures between the groups were combined into a principal component and included in a logistic regression model to evaluate prediction accuracy. Fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity, and mean diffusivity of the disconnected WM tracts were analyzed.

Results Baseline structural disconnections in the mid-posterior and central corpus callosum predicted poor functional outcomes at 3 months, and increased somatomotor network (SMN) disconnection severity correlated with poor recovery. Age, National Institutes of Health Stroke Scale score, and structural disconnections significantly predicted functional outcomes in logistic regression models. The first principal component analysis of the dysconnectivity measures explained 88% of the total variance and improved prediction accuracy from 53.8% to 76.9%. Differences in FA and RD in the region of interest of the corpus callosum between outcome groups were statistically significant.

Conclusions Predictive outcome markers from probabilistic structural disconnection mapping in acute stroke emphasize preserving interhemispheric corpus callosum and SMN connections.

Keywords Stroke; Magnetic resonance imaging; Connectivity; Damage; Outcome

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Introduction

Stroke is a leading cause of death and disability worldwide, affecting a quarter of the population who are expected to experience at least one episode in their lifetime.¹ Common disabilities include sensorimotor and cognitive impairments, which need to be addressed through rehabilitation therapy.² While clinical evidence supports certain parameters, such as National Institutes of Health Stroke Scale (NIHSS) scores and infarct volume, in predicting functional outcomes after an acute ischemic stroke,³ the uncertainty in the response of some patients, especially those with intermediate clinical deficits after reperfusion treatment, highlights the need for complex analytical methodologies that consider structural and functional connectivity parameters. These methodologies shift the focus toward a more comprehensive perspective of the brain as a complex and interconnected network system. Structural and functional connectivity play a crucial role in determining the severity of brain damage, which can affect a patient's response to treatment and outcomes in the first few months following a stroke. Brain lesions often cause nonlocal effects, and several studies suggest that post-stroke deficits result from physiological dysfunction in distributed brain networks rather than being limited to the lesion site alone.

Variability in stroke lesion location and size complicates spatial normalization⁴ and impedes voxel-based statistical models, rendering traditional methods ineffective. Nevertheless, the indirect estimation of structural connectivity damage has been shown to accurately predict behavioral deficits after stroke.⁵

In this study, we applied a probabilistic methodology⁶ to quantify brain damage and structural disconnections in the white matter (WM) that affect cortical areas, using lesion masking on a tractography atlas and parcellation of gray matter into functional network nodes. We aimed to determine whether disconnection markers can predict functional outcomes after acute ischemic stroke. Furthermore, we validated the results using individual diffusion tensor imaging (DTI) measurements.

Methods

Patients

This prospective longitudinal study was a sub-study of the BRAIN-CONNECTS study that included consecutive patients aged >18 years who were admitted to our stroke unit with first-ever ischemic stroke between September 2017 and May 2020. Patients had moderate impairment, defined as NIHSS scores between 5 and 13, and were able to safely tolerate magnetic resonance imaging (MRI) scanning and behavioral testing. All patients had a modified Rankin Scale (mRS) of zero on admission and underwent

clinical follow-up at 90 days. Patients received intravenous recombinant tissue plasminogen activator and were treated with mechanical thrombectomy according to the guidelines.⁷ Patients with primary intracranial hemorrhage, symptomatic hemorrhagic transformation, new infarcts after the initial stroke, or absolute contraindications to MRI were excluded. Stroke severity was defined using the NIHSS score at day 3. Functional outcome at 90 days was assessed using the mRS; patients with mRS ≤ 2 were considered functionally independent.⁸ Standard rehabilitation was initiated once patients were clinically stable. The patients were divided into two groups: those with significant functional improvement (SFI) and those with no significant functional change (NSFC) at the 3-month follow-up. SFI was defined as the change from an mRS score of 3–5 at discharge to 0–2 at 3 months. Patients with an mRS score of 0–2 at discharge whose scores did not change at 3 months were excluded. Patients who had a score of 3–5 at discharge and who persisted in this range at 3 months follow-up were categorized into the NSFC group. The study was approved by the institutional ethics committee (Comitè Ètic d'Investigació Clínica del Hospital Universitari de Girona Doctor Josep Trueta; protocol code, BRAIN-CONNECTS; reference number, 2017.099; approval date, July 2017) and conducted in accordance with the Declaration of Helsinki. All participants or their relatives provided written informed consent prior to participating in the study.

MRI acquisition

All scans were acquired on day 3 after symptom onset using a 1.5-T MRI system (Ingenia system; Philips Medical Systems, Best, The Netherlands) with a 15-channel phased-array head coil, with foam padding and headphones used to restrict head motion and reduce scanner noise, respectively. The scan protocol included axial T1-weighted images (repetition time [TR]: 8.4 ms; echo time [TE]: 4.1 ms; flip angle: 8°; field of view [FOV]: 230 mm; voxel size: 1×1×1 mm). Axial DTI was performed in 32 non-collinear diffusion directions, with b-values of 0 and 1,000 s/mm², using the echo planar imaging protocol (TR: 4,470 ms; TE: 93 ms; flip angle: 90°; FOV: 230 mm; no gap; voxel size: 1.8×1.8×2 mm). Diffusion-weighted images (DWI) were acquired with b-values of 0 and 1,000 seconds/mm² (TR: 3,000 ms; TE: 87 ms; FOV: 230×230 pixel matrix; voxel size: 1×1×5 mm³).

Lesion mapping and preprocessing

Segmentation of the stroke lesion volumes of interest (VOI) was performed using Imfusion Labels (version 0.18; ImFusion GmbH, Munich, Germany) on the b1000 DWI sequence. Lesion VOIs were obtained semi-automatically and reviewed by a radiologist with more than 20 years of experience. Finally, individual lesion masks

were then resliced to a grid size of 182×218×182 at 1-mm resolution to match the Montreal Neurological Institute (MNI)-space template for lesion quantification analysis.

DTI preprocessing

The diffusion data were reconstructed in MNI space using q-space diffeomorphic reconstruction to obtain the spin distribution function.⁸ A diffusion sampling length ratio of 1.25 was used, and the output resolution in the diffeomorphic reconstruction was 2-mm isotropic. Restricted diffusion was quantified using restricted diffusion imaging,^{9,10} and tensor metrics were calculated using DWI with b-values lower than 1,750 s/mm. Subsequently, fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) maps were computed. All the steps were performed using DSI Studio (version Chen Feb 5, 2024; <https://dsi-studio.labsolver.org>).

Structural dysconnectivity measures

Structural connectivity measures were calculated using the Lesion Quantification Toolkit (LQT) in R implementation (version 0.1.0; R Foundation for Statistical Computing, Vienna, Austria; Dworkin 2020). By incorporating the normalized lesion mask for each subject, we obtained damage and dysconnectivity measures for 135 gray matter parcellations based on a 100-parcel parcellation¹¹ (augmented with 35 subcortical and cerebellar regions, including the brainstem)⁶ and 70 WM tracts from the HCP-842 curated tract segmentation.¹² These measures included parcellation damage, tract disconnection, regional disconnection, parcellation-to-parcellation disconnection, network disconnection, and network-to-network disconnection. Parcellation damage reflects the percentage of voxels in each atlas region that fall within the lesion mask, whereas disconnection measures account for the number of affected fiber streams in the WM regions of the HCP-842 atlas. Disconnection severity for gray matter regions was calculated either for a single region or between pairs of regions, considering the end-to-end connectogram. The net disconnection severity was calculated by considering the membership of the affected gray matter regions based on a 7-network atlas.¹¹

Statistical analysis

We reduced the dimensionality of the disconnection data by including only variables representing brain regions that affected a minimum of 10% in at least half of the cohort and applied two-sample t-tests or Wilcoxon rank-sum tests (according to data distribution) to detect differences between groups. A significance threshold of $P < 0.050$ was used, and a False Discovery Rate (FDR) adjustment was applied to control for multiple com-

parisons. To account for potential correlations with outcomes, logistic regression models were computed for each significantly different disconnection measure. As these variables were not normally distributed, a Box-Cox transformation was applied. However, we found collinearity among the significantly differing disconnection measures. Therefore, we performed principal component analysis (PCA) to create a new variable that combined the identified measures to determine the degree of contribution of each measure to population variance. The accuracy of the logistic regression models was evaluated using a two-pronged approach, and initial validation was performed by dividing the sample into training (67% of samples) and testing sets. To ensure model stability and provide a robust estimate, a bootstrap resampling method ($R = 2,000$ replicates) was employed to estimate model accuracy and its confidence interval. All analyses and tests were conducted using R software (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria).

In-patient DTI measures

To further validate the lesion quantification results, we examined differences in individual patient DTI measures between the groups. First, we used a deterministic approach to assess the FA, AD, RD, and MD, respectively, of the increased disconnected tracts in the NSFC group. The region of interest (ROI) was defined by seeding the Desikan-Killiany atlas regions corresponding to the disconnected WM tracts to obtain fiber projections in the International Consortium for Brain Mapping (ICBM) template using DSI Studio. Mean ROI values were extracted by masking individual DTI maps with the resulting ROI using an in-house MATLAB (MathWorks Inc., Natick, MA, USA) script. Differences in FA, AD, RD, and MD between groups were tested and FDR-corrected using R.

Second, the same analysis was performed within the DTI space using a probabilistic approach, specifically correlational tractography in DSI Studio, by correlating diffusion MRI quantitative anisotropy (QA) with the functional outcome group. A non-parametric Spearman partial correlation was used to derive the correlation, with the effects of age and NIHSS score at discharge removed using a multiple regression model. A T-score threshold of 2.5 was assigned and tracked using a deterministic fiber tracking algorithm¹³ to obtain correlational tractography. The same ROI used in the deterministic approach served as the seeding region for the whole brain. Tracks were filtered using topology-informed pruning¹² for 16 iterations. An FDR threshold of 0.05 was applied to select tracks. To estimate the FDR, 4,000 randomized permutations were applied to the group labels to obtain the null distribution of track length.

Results

Ischemic lesions in 43 patients were segmented and included in the analysis. The sociodemographic variables, lesion features, and clinical scores are presented in Table 1. Significant differences between functional outcome groups were observed for lesion volume, lesion location, and subacute NIHSS score. The probabilistic distributions of lesions for all cohorts and groups are shown in Figure 1.

No significant differences in lesion mapping were observed between groups. Average dysconnectivity maps, plotted against the probability of lesions for each group, are presented for illustrative purposes in Figure 2. None of the parcellation damage or net-to-net disconnection variables met the criteria for data di-

dimensionality reduction. We found no statistically significant differences in overall disconnection, between-parcellation disconnections, or in comparison between the SFI and NSFC groups. However, significant probabilistic structural disconnection was found within the mid-posterior (median SFI, 0.206; median NSFC, 8.502; Wilcoxon rank-sum test, $P=0.013$) and central (median SFI, 0.665; median NSFC, 10.314; Wilcoxon rank-sum test, $P=0.022$) regions of the corpus callosum in the NSFC group (Figure 3 and Table 2). Additionally, increased disconnection severity was observed within the somatomotor network (SMN) in patients with NSFC 3 months after discharge (SFI mean, 0.114; NSFC median, 0.176; Wilcoxon rank-sum test, $pFDR=0.039$).

Logistic regression analysis was performed to predict 3-month functional outcomes based on changes in the mRS score from

Table 1. Characteristics of the cohort

	n	All patients	n _{SFI}	SFI patients	n _{NSFC}	NSFC patients	P
Age (yr)	43	76 (61, 81.5)	18	66.56±14.57	25	78 (65, 82)	0.070 [†]
Sex							0.898 [†]
Female	16		6		10		
Male	27		12		15		
Infarct volume	43	8.62 (3.04, 41.54)	18	8.26 (2.53, 35.38)	25	8.62 (5.66, 50.18)	0.297 [†]
Lesioned hemisphere							0.242 [†]
Right	20		6		14		
Left	21		10		11		
Both	1		1		0		
Lesion location							0.070 [†]
Hemispheric	35		12		23		
Brainstem	6		5		1		
Stroke etiology							0.951 [†]
Cardioembolic	17		7		10		
Atheromatosis	8		3		5		
Undetermined	14		5		9		
Vascular occlusion							0.142 [†]
Middle cerebral artery	35		12		23		
Vertebrobasilar	5		4		1		
Treatment admission							0.296 [†]
None	18		7		11		
rt-PA only	14		4		10		
Mechanical thrombectomy and rt-PA	10		6		4		
NIHSS at discharge	43	9.35±2.79	18	8.10±2.61	25	10.24±2.62	0.011 [*]
NIHSS at 3 months	43	4 (2, 6)	18	2 (2, 4)	25	5.84±3.20	<0.001 [†]
mRS at discharge	43	4 (3, 4)	18	3 (3, 3)	25	4 (4, 4)	<0.001 [†]
mRS at 3 months	43	3 (2, 4)	18	2 (1.25, 2)	25	3 (3, 4)	<0.001 [†]

Descriptive values are shown as the mean±standard deviation for normally distributed variables and the median (IQR) for non-normally distributed variables. SFI, significant functional improvement; NSFC, no significant functional change; rt-PA, recombinant tissue plasminogen activator; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; IQR, interquartile range.

*T-test; [†]Wilcoxon rank-sum test; [‡]Chi-squared test.

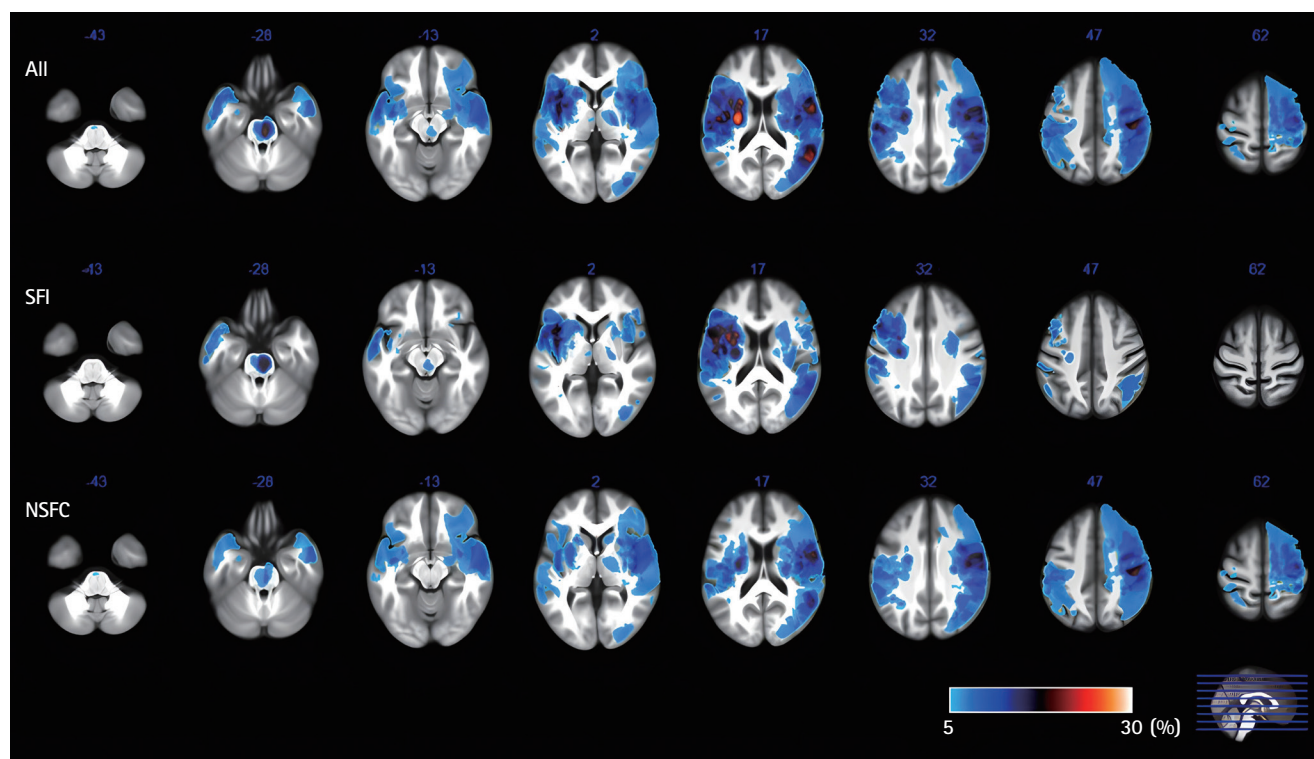


Figure 1. Lesion topography according to the groups. Map values represent the percentage probability of each voxel being within the ischemic lesion for each cohort. SFI, significant functional improvement; NSFC, no significant functional change.

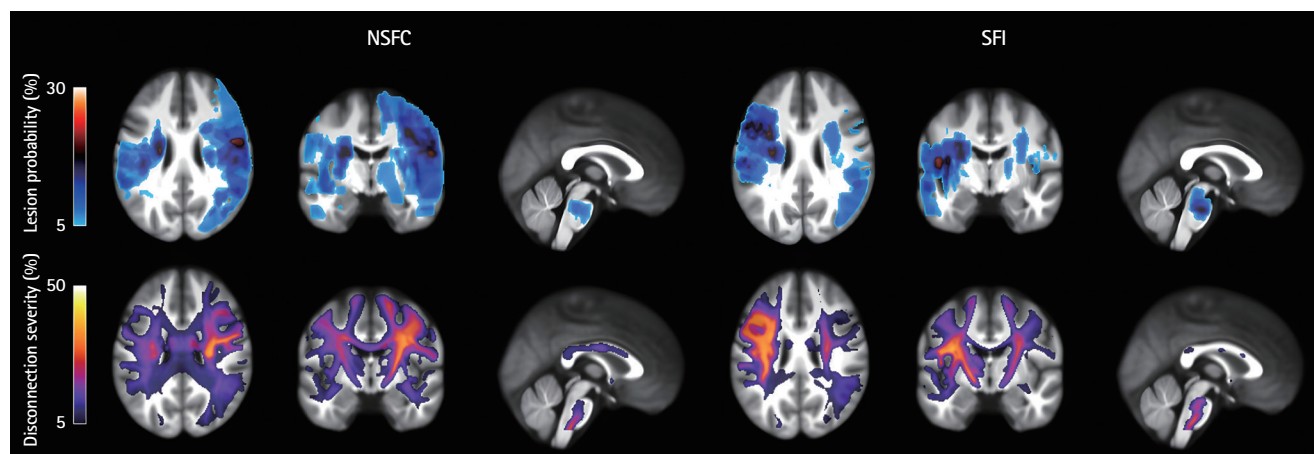


Figure 2. Mean probability of lesion in normalized space and averaged disconnection maps computed with the Lesion Quantification Toolkit for each group. NSFC, no significant functional change; SFI, significant functional improvement.

discharge. The results indicated that age and NIHSS score were significant predictors, whereas sex and lesion volume were not. Additionally, probabilistic structural disconnection severity of the central and mid-posterior parts of the corpus callosum and of the SMN was statistically significant after controlling for baseline NIHSS score and age. The first dimension of PCA, which combined the identified key disconnection measures, accounted for 88% of the total variance and showed a fairly equal contribution from each variable. The prediction accuracy for SFI and NSFC

outcomes was calculated by dividing the dataset into training (67%, 30 samples) and test subsets and adjusting the logistic regression model with age, baseline NIHSS score, and the first PCA component as predictors. The outcome prediction of the null model, which included age and baseline NIHSS scores, showed a prediction accuracy of 53.8% (95% CI 25.1–80.8). This result was further corroborated by bootstrap analysis, which estimated a stable accuracy of 74.4% (95% CI 48.8–83.7). The pseudo- R^2 (Nagelkerke) value of this model was 0.40. The odds ratio

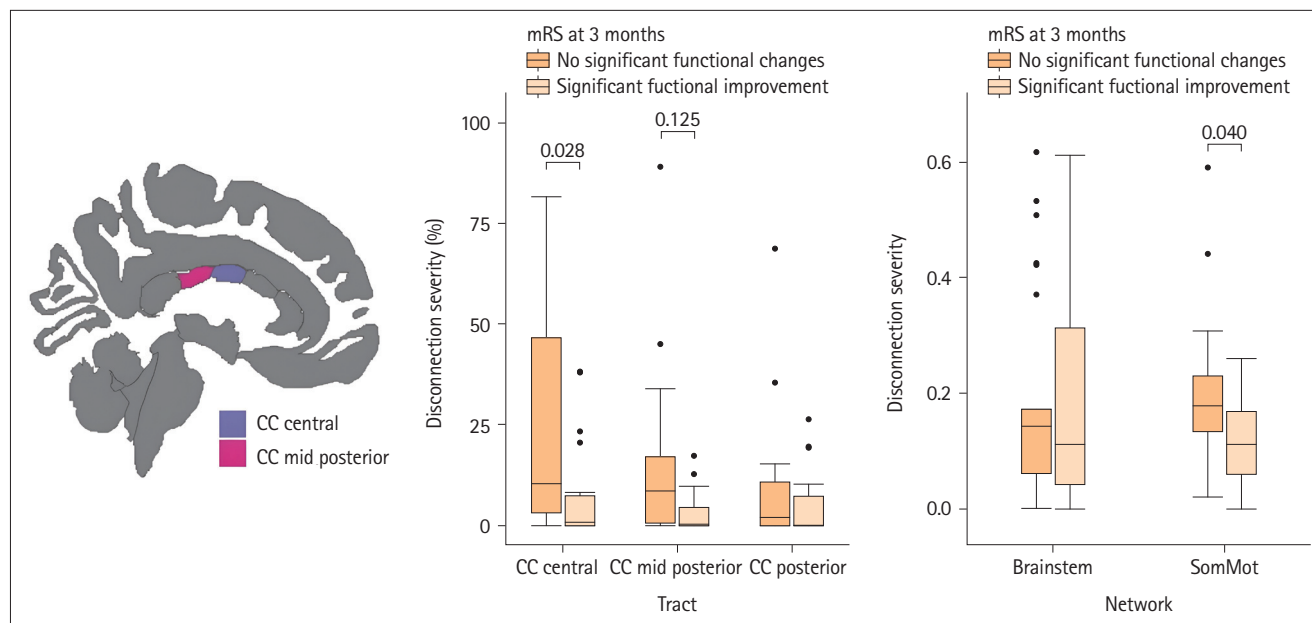


Figure 3. Boxplots of atlas-based structural disconnection severities computed with LQT. Dimensionality reduction of data was applied by selecting only variables representing disconnection in at least half of the cohort with a minimum severity of 10%. For these, group comparisons were computed using two-sample Wilcoxon rank-sum tests, and FDR correction was applied to *P*-values for each test, setting a significance threshold of $P < 0.050$. CC, corpus callosum; mRS, modified Rankin Scale; SomMot, somatomotor; LQT, Lesion Quantification Toolkit; FDR, False Discovery Rate.

for NIHSS was 0.57 (95% CI 0.35–0.81), suggesting that for each one-point increase in NIHSS score, the odds of functional improvement decreased by 43%. Adding the PCA component significantly improved model performance, increasing the prediction accuracy to 76.9% (95% CI 46.2–95.0). Bootstrap analysis confirmed the model's stability, with an estimated accuracy of 81.4% (95% CI 65.1–88.4). The pseudo- R^2 value for the enhanced model was 0.40. The odds ratio for the PCA component was 0.48 (95% CI 0.18–0.92), indicating that for each one-unit increase in this component, the odds of a positive outcome decreased by 52%. In this model, the odds ratio for NIHSS at baseline was 0.66 (95% CI 0.43–0.91), indicating a slightly weaker but still significant association with the outcome compared to the null model.

Of the initial 43 patients with segmented lesion masks, 38 (15 SFI and 23 NSFC) had DTI sequences available for processing. To ensure data reliability, a comprehensive quality control analysis was performed for all DTI acquisitions. None of the participants was identified as an outlier. Motion correction metrics revealed an average DWI correlation of 0.6683 ± 0.0623 , indicating minimal subject movement during scanning, while the number of scans with significant motion or artifacts was low (median=0, interquartile range=0, 0).

The ROI used to assess differences in FA, AD, RD, and MD values was obtained as the sum of bilateral projections of the central and mid-posterior regions of the corpus callosum, masked by the ICBM body of the corpus callosum region to avoid over-

lap with lesional sites, and placed in MNI space. As the variables were normally distributed and showed homoscedasticity between the subsamples, a *t*-test was applied. We found statistically significant differences in the FA and RD between the SFI and NSFC groups (Table 3), even after FDR correction. The FA decreased, whereas RD increased in the NSFC group, suggesting microstructural changes detected using the atlas-based lesion quantification approach. Furthermore, using a probabilistic approach, we identified bilateral projecting tracts of the corpus callosum with statistically lower QA in the NSFC group (FDR-corrected $P < 0.050$), as shown in Figure 4, thus validating our previous findings.

Discussion

Our study found that probabilistic structural disconnection mapping is effective in predicting long-term functional outcomes based on acute stroke disconnections. By incorporating atlas-based structural disconnection parameters, prediction accuracy increased from 53.8% to 76.9%. Although lesion volume is a potential biomarker for predicting stroke recovery outcomes, it was not a strong predictor in our sample. The PCA results suggest that the identified disconnection measures are highly correlated, with minimal differences in their contributions. We propose that each of these measures represents a predictive marker for poor outcomes at the 3-month follow-up, as they appear to be concurrent and reflect the same disruption pathway in our

Table 2. Dysconnectivity measures computed with the Lesion Quantification Toolkit

	n	All patients	n _{SFI}	SFI patients	n _{NSFC}	NSFC patients	P	pFDR
Tract disconnection								
CC central	43	4.510 (0, 22.218)	18	0.665 (0, 7.514)	25	10.314 (3.253, 46.543)	0.015	0.021 [†]
CC mid posterior	43	4.010 (0, 13.127)	18	0.206 (0, 4.373)	25	8.502 (0.749, 17.041)	0.003	0.013 [†]
CC posterior	43	0.495 (0, 9.534)	18	0.004 (0, 7.153)	25	1.877 (0, 10.609)	0.244	0.244 [†]
Parcelation disconnection								
LH_SomMot_5	43	0.183 (0.029, 0.340)	18	0.081 (0.006, 0.286)	25	0.2197±0.1511	0.209	0.631*
LH_DorsAttn_FEF_1	43	0.113 (0, 0.270)	18	0.156 (0, 0.394)	25	0.112 (0, 0.199)	0.814	0.814 [†]
Brainstem	43	0.122 (0.054, 0.300)	18	0.110 (0.042, 0.313)	25	0.142 (0.061, 0.172)	0.805	0.814*
Network disconnection								
Brainstem	43	0.122 (0.054, 0.300)	18	0.110 (0.042, 0.313)	25	0.142 (0.061, 0.172)	0.778	0.778 [†]
SomMot	43	0.137 (0.094, 0.218)	18	0.114±0.075	25	0.176 (0.134, 0.231)	0.019	0.039 [†]
Network-to-network disconnection								
Brainstem_to_DorsAttn	43	0.194 (0.042, 0.291)	18	0.174 (0.034, 0.337)	25	0.194 (0.094, 0.271)	0.901	0.931 [†]
Brainstem_to_SalVentAttn	43	0.162 (0.008, 0.385)	18	0.250 (0.032, 0.430)	25	0.087 (0.005, 0.295)	0.347	0.666 [†]
Brainstem_to_SomMot	43	0.219 (0.012, 0.389)	18	0.152 (0, 0.351)	25	0.253±0.190	0.218	0.666 [†]
Default_to_SomMot	43	0.118 (0.001, 0.320)	18	0.219 (0, 0.393)	25	0.062 (0.006, 0.275)	0.550	0.709 [†]
DorsAttn_to_Striatum	43	0.108 (0.071, 0.159)	18	0.108 (0.004, 0.144)	25	0.119±0.061	0.445	0.666 [†]
DorsAttn_to_Thalamus	43	0.111 (0.014, 0.326)	18	0.069 (0, 0.288)	25	0.125 (0.014, 0.333)	0.387	0.666 [†]
SalVentAttn_to_SomMot	43	0.149 (0.005, 0.289)	18	0.151 (0, 0.319)	25	0.132 (0.011, 0.255)	0.931	0.931 [†]
SomMot_to_Striatum	43	0.151 (0.059, 0.226)	18	0.063 (0, 0.204)	25	0.182±0.098	0.034	0.303 [†]
SomMot_to_Thalamus	43	0.167 (0.017, 0.333)	18	0.133 (0, 0.308)	25	0.225±0.174	0.258	0.666 [†]

Dimensionality reduction of data was applied by selecting only variables representing disconnection in at least half of the cohort for a minimum of 10% of severity. Descriptive values are shown as the mean±standard deviation for normally distributed variables and the median (IQR) for non-normally distributed variables.

SFI, significant functional improvement; NSFC, no significant functional change; FDR, False Discovery Rate; CC, corpus callosum; LH, left hemisphere; SomMot, somatomotor; DorsAttn_FEF, dorsal attention_frontal eye field; SalVentAttn, salience ventral attention; IQR, interquartile range.

*T-test for normally distributed and equal variance variables; [†]Wilcoxon rank-sum test, otherwise.

Table 3. DTI values within the central and mid posterior regions of the corpus callosum

	n	All patients	n _{SFI}	SFI patients	n _{NSFC}	NSFC patients	P	pFDR
Fractional anisotropy	38	0.522±0.032	15	0.542±0.024	23	0.510±0.030	<0.001	0.002
Axial diffusivity	38	1.494±0.080	15	1.489±0.071	23	1.498±0.087	0.738	0.738
Radial diffusivity	38	0.624±0.071	15	0.593±0.058	23	0.644±0.072	0.022	0.044
Mean diffusivity	38	0.914±0.070	15	0.892±0.061	23	0.928±0.073	0.102	0.136

ROI was placed at MNI (39,42,28) and mean ROI values were extracted. Significant differences were tested using t-test and P-values were corrected with the FDR method.

DTI, diffusion tensor imaging; SFI, significant functional improvement; NSFC, no significant functional change; FDR, False Discovery Rate; ROI, region of interest; MNI, Montreal Neurological Institute.

NSFC sample.

These findings highlight the importance of preserving connections between the two brain hemispheres, specifically within the SMN, through the corpus callosum. This suggests that these connections play a crucial role in the recovery process and are potentially compromised in the middle of the corpus callosum. This damage may impede the effectiveness of rehabilitation efforts by disrupting communication between the nodes of the SMN in the opposite hemisphere. Our findings are consistent

with previous studies demonstrating a link between lower inter-hemispheric functional connectivity and poor outcomes in stroke patients,^{3,14-17} particularly within the SMN. Additionally, behavioral deficits have been associated with reduced functional connectivity in the corresponding networks, as observed in patients with a loss of interhemispheric connectivity in the motor network.¹⁸

This finding is also consistent with the decreased FA and increased RD in the corpus callosum observed in patients with

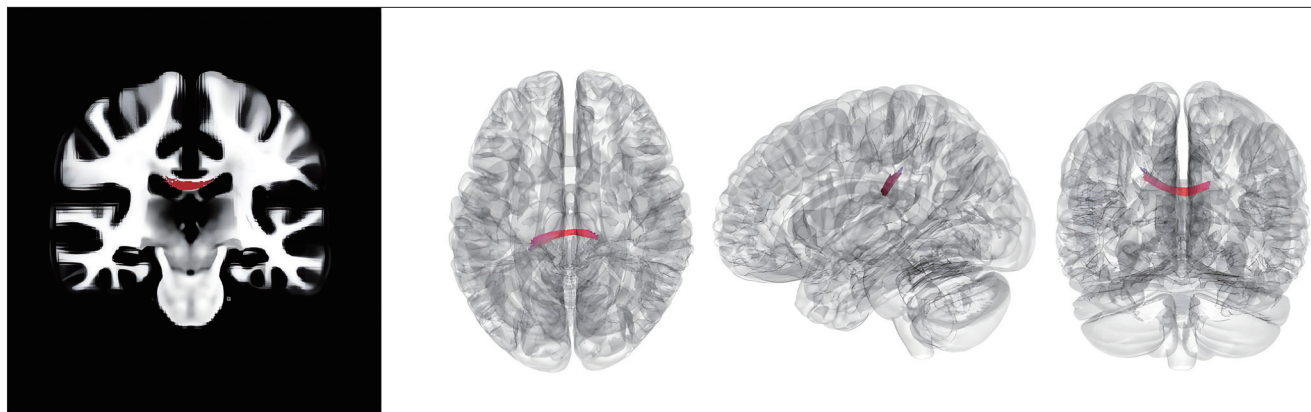


Figure 4. Correlational tractography results. Diffusion MRI quantitative anisotropy was correlated with the functional outcome group using Spearman's partial correlation, controlling for the effects of age and NIHSS at discharge. Fibers of corpus callosum are displayed in red. An FDR-corrected *P*-value threshold of 0.050 was applied. MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; FDR, False Discovery Rate.

poor outcomes in our cohort, suggesting axonal loss within this region, which connects the interhemispheric SMN nodes, even when the ischemic lesion occurs at a distant site. Many previous studies have focused on microstructural damage in the corticospinal tract as a WM motor pathway, but few have included the corpus callosum in their analyses.^{19,20} Similar to our study, Li et al.¹⁹ previously found significantly decreased callosal FA and increased RD and AD in stroke patients compared to controls. Stewart et al.²⁰ also found a positive correlation between FA in the premotor section of the corpus callosum and reaching performance. However, to the best of our knowledge, this is the first study to link atlas-based probabilistic structural disconnections with deterministic DTI microstructure measures for predicting 3-month outcomes after acute stroke. Knowing the degree of structural disconnection in the first few days after a stroke can help in designing personalized rehabilitation programs. Therefore, patients with a more severe degree of disruption may benefit from a more intensive rehabilitation strategy.

Our analysis required only standard lesion mapping from routinely acquired DWI. However, this study has several limitations, including a small sample size and the use of a standardized structural connectome atlas instead of whole-brain tractography to infer structural disconnections. This approach was used because the reconstruction of WM fibers from diffusion tensor images is unreliable in the presence of brain lesions. Although we did not account for individual variability or discrepancies between our cohort and the population represented in the HCP atlas in the lesion quantification analysis, our findings were consistent with microstructural changes computed using the BRAIN-CONNECTS DTI sequence in a non-lesioned site, such as the corpus callosum, indicating that LQT disconnection metrics are sufficiently robust to be validated with a 32-direction DTI acquisition. However, 32-direction DTI images, as used in this

study for validation, have lower angular resolution compared with the 90 directions of the atlas used, possibly underestimating brain damage in crossing-fiber regions accounted for by the atlas. Further research is required to validate this tool and determine whether it can replace expensive, time-consuming sequences for assessing the microstructural damage of interhemispheric connections. Patients with NIHSS scores between 5 and 13 face more uncertainty regarding outcomes than those with lower scores, who are more likely to have favorable outcomes at 3 months according to the Rankin Scale, and those with scores above 13, who usually have poor outcomes. Addressing this uncertainty is the main goal of BRAIN-CONNECTS. Therefore, one limitation of this study is that the results cannot be generalized to patients with other stroke severity levels or beyond 3 months post-onset. Future studies should examine how age-related WM changes and rehabilitation intensity affect outcomes in patients with moderate disease severity.

Conclusions

This study highlights the role of structural connectivity, particularly within the corpus callosum, in predicting stroke recovery. Probabilistic structural disconnection mapping provides valuable predictive markers for WM tract damage and poststroke outcomes. These findings emphasize the importance of preserving interhemispheric connections within the SMN and suggest that structural disconnections in the acute phase serve as significant indicators of mid- and long-term functional outcomes.

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Conflicts of interest

The authors have no financial conflicts of interest.

Author contribution

Data curation: Elena de la Calle, Carles Biarnés. Formal analysis: Elena de la Calle, Carles Biarnés. Funding acquisition: Josep Puig, Salvador Pedraza. Investigation: Josep Puig, Esther Duarte, Andrea Morgado-Pérez, Mikel Terceño, Yolanda Silva, Santiago Medrano, Jaume Capellades, Salvador Pedraza, Anira Escrichs, Pepus Daunis-i-Estadella, Marc Comas-Cufí, Luca Saba, Kambiz Nael, Víctor Pineda. Methodology: Josep Puig, Elena de la Calle, Carles Biarnés, Marian Martí-Navas, Esther Duarte, Pepus Daunis-i-Estadella, Marc Comas-Cufí, Mikel Terceño. Supervision: Josep Puig, Salvador Pedraza, Carles Biarnés, Marian Martí-Navas. Writing—original draft: Elena de la Calle, Carles Biarnés, Josep Puig. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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References

- Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World Stroke Organization (WSO): global stroke fact sheet 2022. *Int J Stroke* 2022;17:18–29.
- Morgado-Pérez A, Coll-Molinós M, Valero R, Llobet M, Rueda N, Martínez A, et al. Intensive rehabilitation program in older adults with stroke: therapy content and feasibility—preliminary results from the BRAIN-CONNECTS study. *Int J Environ Res Public Health* 2023;20:4696.
- Puig J, Blasco G, Alberich-Bayarri A, Schlaug G, Deco G, Biarnés C, et al. Resting-state functional connectivity magnetic resonance imaging and outcome after acute stroke. *Stroke* 2018;49:2353–2360.
- Crinion J, Ashburner J, Leff A, Brett M, Price C, Friston K. Spatial normalization of lesioned brains: performance evaluation and impact on fMRI analyses. *Neuroimage* 2007;37:866–875.
- Salvalaggio A, De Filippo De Grazia M, Zorzi M, Thiebaut de Schotten M, Corbetta M. Post-stroke deficit prediction from lesion and indirect structural and functional disconnection. *Brain* 2020;143:2173–2188.
- Griffis JC, Metcalf NV, Corbetta M, Shulman GL. Lesion quantification toolkit: a MATLAB software tool for estimating grey matter damage and white matter disconnections in patients with focal brain lesions. *Neuroimage Clin* 2021;30:102639.
- Turc G, Bhogal P, Fischer U, Khatri P, Lobotesis K, Mazighi M, et al. European Stroke Organisation (ESO) – European Society for Minimally Invasive Neurological Therapy (ESMINT) guidelines on mechanical thrombectomy in acute ischemic stroke. *J Neurointerv Surg* 2023;15:e8.
- Saver JL, Chaisinanunkul N, Campbell BCV, Grotta JC, Hill MD, Khatri P, et al. Standardized nomenclature for modified Rankin Scale global disability outcomes: consensus recommendations from Stroke Therapy Academic Industry Roundtable XI. *Stroke* 2021;52:3054–3062.
- Yeh FC, Tseng WY. NTU-90: a high angular resolution brain atlas constructed by q-space diffeomorphic reconstruction. *Neuroimage* 2011;58:91–99.
- Yeh FC, Liu L, Hitchens TK, Wu YL. Mapping immune cell infiltration using restricted diffusion MRI. *Magn Reson Med* 2017;77:603–612.
- Schaefer A, Kong R, Gordon EM, Laumann TO, Zuo XN, Holmes AJ, et al. Local-global parcellation of the human cerebral cortex from intrinsic functional connectivity MRI. *Cereb Cortex* 2018;28:3095–3114.
- Yeh FC, Panesar S, Barrios J, Fernandes D, Abhinav K, Meola A, et al. Automatic removal of false connections in diffusion MRI tractography using topology-informed pruning (TIP). *Neurotherapeutics* 2019;16:52–58.
- Yeh FC, Verstynen TD, Wang Y, Fernández-Miranda JC, Tseng WY. Deterministic diffusion fiber tracking improved by quantitative anisotropy. *PLoS One* 2013;8:e80713.
- Min YS, Park JW, Park E, Kim AR, Cha H, Gwak DW, et al. Interhemispheric functional connectivity in the primary motor cortex assessed by resting-state functional magnetic resonance imaging aids long-term recovery prediction among subacute stroke patients with severe hand weakness. *J Clin Med* 2020;9:975.
- Carter AR, Astafiev SV, Lang CE, Connor LT, Rengachary J, Strube MJ, et al. Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke. *Ann Neurol* 2010;67:365–375.
- van Meer MP, van der Marel K, Wang K, Otte WM, El Bouazati S, Roeling TA, et al. Recovery of sensorimotor function after experimental stroke correlates with restoration of resting-state interhemispheric functional connectivity. *J Neurosci* 2010;30:3964–3972.
- Park CH, Chang WH, Ohn SH, Kim ST, Bang OY, Pascual-Leone A, et al. Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. *Stroke* 2011;42:1357–1362.

18. Baldassarre A, Ramsey L, Rengachary J, Zinn K, Siegel JS, Metcalf NV, et al. Dissociated functional connectivity profiles for motor and attention deficits in acute right-hemisphere stroke. *Brain* 2016;139:2024–2038.
19. Li Y, Wu P, Liang F, Huang W. The microstructural status of the corpus callosum is associated with the degree of motor function and neurological deficit in stroke patients. *PLoS One* 2015;10:e0122615.
20. Stewart JC, O'Donnell M, Handlery K, Winstein CJ. Skilled reach performance correlates with corpus callosum structural integrity in individuals with mild motor impairment after stroke: a preliminary investigation. *Neurorehabilitation and Neural Repair* 2017;31:657–665.