A template-based procedure for determining white matter integrity in the internal capsule early after stroke

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The integrity of descending white matter pathways, measured by fractional anisotropy from DW-MRI, is a key prognostic indicator of motor recovery after stroke. Barriers to translation of fractional anisotropy measures into routine clinical practice include the time required for manually delineating volumes of interest (VOIs), and inter-examiner variability in this process. This study investigated whether registering and then editing template volumes of interest ‘as required’ would improve inter-examiner reliability compared with manual delineation, without compromising validity. MRI was performed with 30 sub-acute stroke patients with motor deficits (mean NIHSS = 11, range 0–17). Four independent examiners manually delineated VOIs for the posterior limbs of the internal capsules on T1 images, or edited template VOIs that had been registered to the T1 images if they encroached on ventricles or basal ganglia. Fractional anisotropy within each VOI and interhemispheric asymmetry were then calculated. We found that 13/30 registered template VOIs required editing. Edited template VOIs were more spatially similar between examiners than the manually delineated VOIs (p = 0.005). Both methods produced similar asymmetry values that correlated with clinical scores with near perfect levels of agreement between examiners. Contralesional fractional anisotropy correlated with age when edited template VOIs were used but not when VOIs were manually delineated. Editing template VOIs as required is reliable, increases the validity of fractional anisotropy measurements in the posterior limb of the internal capsule, and is less time-consuming compared to manual delineation. This approach could support the use of FA asymmetry measures in routine clinical practice.

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1. Introduction

Diffusion tensor imaging provides information about tissue composition that reflects the microstructural integrity of white matter tracts in the brain (Basser, 1995; Basser and Pierpaoli, 1996). Fractional anisotropy (FA) quantifies the extent to which water diffusion is directionally restricted and is the most common DTI parameter used to assess white matter integrity (Jang, 2010). Disruption of white matter tracts results in less restriction on water diffusion and a lowering of the FA value (Basser and Pierpaoli, 1996; Werring et al., 2000). After stroke, reduced white matter integrity can occur acutely within the primary lesion location, and can be a delayed and distant consequence of anterograde and/or retrograde axonal degeneration (Thomalla et al., 2004; Werring et al., 2000). FA derived from DTI correlates with upper limb function in chronic stroke patients (Stinear et al., 2007), and can be used to predict recovery of upper limb motor function at both early (Jang et al., 2005, 2008; Maeda et al., 2005; Stinear et al., 2012) and chronic stages of stroke (Stinear et al., 2007).

Two approaches to DTI-based analysis can be used to evaluate FA values in white matter tracts. The first is white matter tractography, in which three-dimensional reconstructions of tract trajectories are calculated from the DTI vector field (Mori and van Zijl, 2002). Regions of interest are defined, typically on a single axial slice, as seeds, waypoints and endpoints for subsequent tractography. Mean FA values along the tracts can then be compared between contralesional and ipsilesional hemispheres (Borich et al., 2012; Puig et al., 2012; Wakanaka et al., 2007). This approach can be confounded by anatomical variations or stroke lesions, and requires at least a part of the tract of interest to be intact from start to finish (Borich et al., 2012; Puig et al., 2011; Tang et al.,...
A second approach involves defining a three-dimensional VOI and then calculating the mean FA within the volume. A typical choice of VOI is the posterior limb of the internal capsule (PLIC). Mean FA is calculated bilaterally within the PLIC VOIs to determine FA asymmetry (Borich et al., 2012; Stinear et al., 2007). FA asymmetry correlates with current upper limb motor function (Lindenberg et al., 2010; Zhu et al., 2010), and can be used as a predictor of motor recovery in both chronic (Stinear et al., 2007) and acute patients (Jang et al., 2005; Stinear et al., 2012).

The most commonly used method to define VOIs is manual tracing, in which experienced examiners delineate anatomical structures (Karnath and Perenin, 2005; Moro et al., 2008; Mort et al., 2003). Manual drawing methods remain the gold standard for the exact delineation of anatomical structures as they require fewer computing resources than tractography-based approaches and are more intuitive for clinical use. However, shortcomings of manual tracing include: being limited to regions identifiable by anatomic landmarks (Eckert et al., 2008); being labor intensive (Ashton et al., 2003; Seghier et al., 2008); and erroneous inclusion of structures such as gray matter and other tracts (Holodny et al., 2005; Park et al., 2008). For these reasons, an automated method of VOI delineation may be preferable. Automated methods are much faster, and they may also minimize inter-examiner disagreements (Wilke et al., 2011). However, automated methods may have shortcomings for studies of stroke patients, such as inadequate compensation for the structural distortions introduced by lesions that may result in gray matter or CSF being included in the VOI. Additionally, there may be inadequate correction for anatomical variability between subjects (Fiez et al., 2000). A compromise between manual and automated approaches exists in which VOIs produced by an automated technique are manually edited as required to correct any inappropriate inclusion of gray matter or CSF. It is not clear whether manual or edited VOI delineation methods produce measures that are the more reliable and accurate.

The aims of this study were to compare the reliability and validity of manual and edited VOI methods for defining the PLIC on MR images from sub-acute stroke patients. We hypothesized that editing registered templates would produce valid and more reliable FA values, compared to the more time-consuming process of manual delineation.

2. Material and methods

2.1. Participants

Participants were recruited if they were at least 18 years old and had experienced a first-ever ischemic stroke resulting in persistent unilateral upper limb impairment. Exclusion criteria were any neurological or other conditions that would prevent informed consent or hinder the acquisition or interpretation of the data, such as cognitive or communication deficits, previous stroke, and contra-indications to MRI. Participants were screened using a MRI safety checklist. The study was approved by the regional ethics committee, and all participants provided written informed consent, in accordance with the Declaration of Helsinki.

There were 30 participants in this study (20 females; mean age 68 years, range 31 to 92 years; 19 with right hemisphere lesions and 11 with left hemisphere lesions; Table 1, Fig. 1) and all except one was right-hand dominant before their stroke. All participants completed MR imaging within a mean of 11 (range 4 to 22) days of stroke. At a mean of 13 (range 5 to 23) days after stroke, a clinical assessor evaluated stroke severity using the National Institutes of Health Stroke Scale (NIHSS), motor impairment of the affected upper limb using the Fugl-Meyer (FM) scale, maximum score 66 (Fugl-Meyer et al., 1975); and upper limb function using the Action Research Arm Test (ARAT), maximum score of 57 (Lyle, 1981). The clinical assessor was blinded to the MR images.

2.2. MR data acquisition

Scanning was performed using a Siemens Magnetom Avanto 1.5 T MRI system. To provide anatomical reference, T1-weighted images were obtained with a 3D MP-RAGE sequence (TR = 11 ms, TE = 4.94 ms, field-of-view = 256 mm and voxel dimensions of 1.0 × 1.0 × 1.0 mm) aligned to an axial plane parallel to the anterior and posterior commissures (the AC–PC line). Diffusion tensor imaging was conducted with a single shot spin echo EPI pulse sequence (factor = 128, TR = 6700 ms, TE = 101 ms, field-of-view = 230 mm and voxel dimensions of 1.8 × 1.8 × 3.0 mm) with 30 uniformly distributed (Stejskal and Tanner, 1965) motion-probing gradient orientations ($\beta = 2000$ s/mm$^2$). Head movement was constrained with expandable foam cushions. MR images were visually inspected for motion artifact or instrumental noise. Scanning was repeated if major artifacts were present. Overall time in the scanner was approximately 20 min per participant.

2.3. Manual PLIC delineation

Each examiner pre-processed the images and manually delineated the PLIC VOIs using FSL (FMRI Software Library, Oxford) (Smith et al., 2004; Woolrich et al., 2009). Four independent examiners (expert: V.K.; examiner 1: M.P.; examiner 2: C.Z.; examiner 3: E.V.) performed cross-sectional VOI delineation of each PLIC. V.K. is a neurologist with clinical experience in interpreting MR images, and hence was deemed to be an ‘expert’ examiner for the purposes of this study. The other 3 ‘novice’ examiners were medical researchers who had previous experience in identifying the pertinent structures. None of the examiners had prior experience in VOI drawing and so were trained in the use of the software packages and the required workflow.

Examiners used the T1-weighted images to delineate the PLICs and no other images were consulted while drawing in order to prevent any bias that FA maps could introduce when determining the PLIC borders. Training for the VOI drawing task was accomplished using previously delineated examples in reference atlases of healthy brains.
Examiners were blinded to the other examiners’ evaluations and to other marked-up scans of the same patient that they may have previously processed. Delineation of the PLICs was performed in both the ipsilesional and contralesional hemisphere, in a voxel-wise fashion using the drawing tools in FSLView. Examiners worked on the axial plane and began at the inferior slice that best corresponded to the AC–PC line. In the case of complete destruction of the ipsilesional PLIC, the region was estimated as a mirror volume to the contralesional PLIC (Stinear et al., 2007). The PLIC typically occupied 20 or more image slices but examiners manually delineated the PLIC on the 10 most inferior slices to allow images to be processed in a timely manner.

2.4. Registration of template PLIC VOIs

Each examiner first skull stripped the structural T1-weighted images using the Brain Extraction Tool (BET) (Smith, 2002). This involved iteratively specifying a skull strip threshold value, visually confirming that the BET process had been completed satisfactorily, and repeating if necessary. DWIs were also skull stripped using BET, using the same iterative procedure.

The Johns Hopkins University (JHU) DTI-based atlas of white matter tracts (Hua et al., 2008; Mori et al., 2008; Wakana et al., 2007) was used to create the PLIC templates. FMRIB’s Linear Image Registration Tool (Smith et al., 2004) was then used to perform a linear transform of the

Table 1

| Subject | Sex | Age (y) | DH | SH | Lesion location | TSS (d) | NIH-SS (/42) | ARAT (/57) | FM (/66) | Edit |
|---------|-----|---------|----|----|----------------|--------|-------------|------------|---------|------|
| 1       | F   | 52      | R  | R  | BS             | 21     | 2           | 19         | 21      | N    |
| 2       | F   | 39      | R  | R  | CR             | 18     | 3           | 19         | 37      | N    |
| 3       | M   | 68      | R  | L  | PLIC, Thal, BG | 6      | 1           | 56         | 56      | N    |
| 4       | F   | 83      | R  | R  | CR             | 10     | 7           | 21         | 29      | N    |
| 5       | M   | 73      | R  | R  | CR             | 11     | 1           | 51         | 62      | N    |
| 6       | F   | 73      | R  | R  | CR             | 5      | 1           | 44         | 39      | Y    |
| 7       | M   | 78      | R  | L  | CR, PLIC, ALIC, GenIC, Thal, Put, CN, EC | 7 | 6 | 3 | 7 | Y |
| 8       | M   | 64      | R  | R  | CR             | 18     | 11          | 3          | 8       | Y    |
| 9       | M   | 80      | R  | R  | PLIC, Thal, CN | 5      | 1           | 53         | 59      | Y    |
| 10      | M   | 48      | R  | L  | CR             | 7      | 6           | 34         | 43      | Y    |
| 11      | M   | 61      | R  | R  | CR             | 11     | 17          | 3          | 6       | Y    |
| 12      | F   | 67      | R  | R  | BS             | 14     | 4           | 37         | 55      | N    |
| 13      | F   | 83      | R  | R  | CR             | 8      | 8           | 3          | 11      | Y    |
| 14      | F   | 73      | R  | L  | CR, PLIC, Put  | 14     | 2           | 42         | 60      | Y    |
| 15      | F   | 68      | R  | R  | PLIC, Thal, Put| 9      | 2           | 19         | 21      | N    |
| 16      | F   | 77      | L  | L  | PLIC, GenIC    | 11     | 6           | 2          | 11      | N    |
| 17      | F   | 90      | R  | R  | CR, PLIC, Put  | 9      | 1           | 34         | 57      | N    |
| 18      | M   | 58      | R  | L  | PT, FT         | 10     | 2           | 52         | 64      | N    |
| 19      | M   | 53      | R  | R  | CR, PLIC, GenIC, Thal, Put | 7 | 3 | 57 | 64 | Y |
| 20      | F   | 69      | R  | L  | CR, PLIC      | 9      | 3           | 31         | 43      | Y    |
| 21      | F   | 64      | R  | L  | BS             | 15     | 5           | 41         | 51      | N    |
| 22      | M   | 31      | R  | R  | M1             | 11     | 2           | 37         | 54      | N    |
| 23      | F   | 79      | R  | R  | CR, PLIC      | 4      | 5           | 3          | 12      | N    |
| 24      | F   | 72      | R  | R  | M1, PMC, Put, EC, FT | 5 | 12 | 0 | 5 | N |
| 25      | F   | 43      | R  | R  | M1, PMC       | 18     | 11          | 0          | 4       | N    |
| 26      | F   | 83      | R  | R  | CR, PLIC, ALIC, GenIC, Put | 22 | 9 | 0 | 4 | N |
| 27      | F   | 67      | R  | R  | BS             | 9      | 0           | 40         | 61      | Y    |
| 28      | F   | 76      | R  | L  | CR             | 10     | 4           | 42         | 48      | N    |
| 29      | F   | 91      | R  | L  | BS             | 5      | 7           | 41         | 60      | Y    |
| 30      | F   | 71      | R  | L  | BS             | 12     | 9           | 19         | 18      | Y    |
| Mean    |     | 68      |     |     |                | 11     | 5           | 27         | 36      |       |
| Min     |     | 31      |     |     |                | 4      | 0           | 0          | 4       |       |
| Max     |     | 91      |     |     |                | 22     | 17          | 57         | 64      |       |

F = female; M = male; y = years; DH = dominant hand; R = right; L = left; SH = stroke-affected hemisphere; Lesion location: ALIC = anterior limb of the internal capsule; BS = brainstem; CN = caudate nucleus; CR = corona radiata; EC = external capsule; FT = frontal lobe; GenIC = genu of the internal capsule; M1 = primary motor cortex; PLIC = posterior limb of the internal capsule; PMC = premotor cortex; PT = parietal lobe; Put = putamen; Thal = thalamus; TP = temporal lobe; TSS = time since stroke when MRI was performed; d = days; NIHSS = National Institutes of Health Stroke Scale; ARAT = Action Research Arm Test; FM = Fugl-Meyer Upper Limb Scale; Edit = registered templates were edited as they encroached on the ventricles and/or thalamus.

Fig. 2. Examples of PLIC VOIs produced by the expert examiner. A) Registered template VOIs. B) Edited template VOIs. C) Manually delineated VOIs.
Montreal Neurological Institute (MNI152) template to native patient-space and apply that same transform to the PLIC templates. Linear registration was used, instead of non-linear registration; the latter has less clinical utility as it requires lesion masking and takes more time.

Registered template VOIs that encroached on the basal ganglia or ventricles were edited by each of the four examiners. Voxel-wise editing of the template PLIC (overlaid onto the axial view of each T1-weighted image) was performed using the drawing tools in FSLView (Smith et al., 2004; Woolrich et al., 2009). See Fig. 2 for example of PLIC VOIs produced by the expert examiner.

2.5. Spatial similarity analysis

The Dice Similarity Index (DSI) (Dice, 1945) was used to quantify the spatial similarity between the VOIs produced by the expert examiner and each of the non-expert examiners. DSI ranges from 0 to 1 and is calculated to be twice the number of overlapping voxels between two VOIs, A and B, divided by the total number of voxels contained in both VOIs; DSI_{A,B} = 2 \times (A \cap B) / (A \cup B), where \cap is the intersection and \cup is the union. If two VOIs contain the same number of voxels, and 50% of their voxels overlap, this results in a DSI of 0.5.

2.6. Fractional anisotropy

Diffusion-weighted images were corrected for motion and eddy currents before using FMRIB's Diffusion Toolbox to compute diffusion tensors and FA maps. The FA maps were then registered to each participant's T1-weighted image, and masked using the PLIC VOIs produced by the four independent examiners. Mean FA values within the contralesional and ipsilesional VOIs were used to calculate FA asymmetry for each dataset. FA asymmetry values were calculated as: FA asymmetry = (FA_{contralesional} - FA_{ipsilesional}) / (FA_{contralesional} + FA_{ipsilesional}), where FA = mean FA in the PLIC of the contralesional hemisphere and FA = mean FA in the PLIC of the ipsilesional hemisphere, yielding a value between −1.0 and +1.0 for each participant. Zero indicates symmetrical mean FA in the PLICs and positive values indicate relatively reduced mean FA in the ipsilesional PLIC (Stinear et al., 2007). No provisions were made for handedness or sex, as previous research indicates that these factors do not influence FA values within the PLIC (Buchel et al., 2004; Takao et al., 2011; Westerhausen et al., 2007).

2.7. Statistical analysis

The sample size was calculated by determining the minimum number of participants required to detect an intraclass correlation coefficient of at least 0.8 for FA measures. With 4 independent examiners, \( \alpha = 0.05 \) and \( \beta = 0.20 \), a minimum sample of 28 participants was required (Walter et al., 1998).

Spatial similarity between the PLIC VOIs produced by the expert and non-expert examiners was analyzed with a RM-ANOVA of the DSIs, with factors: METHOD (manual, edited), EXAMINER (1, 2, 3) and HEMISPHERE (contralesional, ipsilesional). Mean FA values obtained from manually delineated and edited template VOIs were analyzed with a RM-ANOVA with factors: METHOD (manual, edited), EXAMINER (expert, 1, 2, 3) and HEMISPHERE. FA asymmetry was analyzed with a RM-ANOVA with the factor EXAMINER.

To investigate the inter-examiner reliability of the manually delineated and edited template methods, separate two-way random effects, absolute agreement intraclass correlation coefficients (ICC) for single measures were calculated using the mean FA values from the ipsi- and contralesional hemispheres and the FA asymmetry values. According to accepted criteria, ICC values of 0.11–0.2 are considered “slight” agreement, 0.21–0.4 are “fair”, 0.41–0.60 are “moderate”, 0.61–0.80 are “substantial”, and 0.81–1.0 are “almost perfect” agreement (Landis and Koch, 1977).

The ecological validity of the manual and edited VOI delineation methods was evaluated by calculating a simple linear regression between the contralesional FA, measured by each examiner, with age to see if the correlations were negative, as expected. Further validation was performed by calculating separate linear regressions between FA asymmetry and ARAT score, FM score, and NIHSS score. We expected each of these regressions to be significant, as correlations between motor deficit and FA asymmetry have been reported previously (Lindenberg et al., 2010; Puig et al., 2011; Stinear et al., 2007).

Statistical analyses were conducted using SPSS software (20.0 SPSS Inc., Chicago, USA). Statistical results were deemed significant if \( p < 0.05 \). Greenhouse–Geisser corrections were undertaken when sphericity was violated.

3. Results

The registered template VOIs were found to encroach on basal ganglia structures, ventricles or both in 13 of the 30 images. The examiners independently edited these templates, prior to calculation of mean FA values, FA asymmetry, and Dice Similarity Indices. The edited template VOIs were analyzed with a RM-ANOVA of the DSIs, with factors: METHOD (manual, edited), EXAMINER (1, 2, 3) and HEMISPHERE (contralesional, ipsilesional). Mean FA values were higher in the contralesional PLIC VOI of each hemisphere, when they manually delineated the VOIs (Table 3). There were negative correlations between FA asymmetry and NIHSS score for 3 of the examiners when they manually delineated the VOIs (Table 3). There were negative correlations between FA asymmetry and ARAT and FM scores, when examiners used both methods of VOI production. There were positive correlations between FA asymmetry and ARAT score, FM score, and NIHSS score. We expected each of these regressions to be significant, as correlations between motor deficit and FA asymmetry have been reported previously (Lindenberg et al., 2010; Puig et al., 2011; Stinear et al., 2007).

Table 2

| Method       | Measure               | FA asymmetry |
|--------------|-----------------------|--------------|
|              | Contralional FA        | Ipsilesional FA |               |
| Manual       | 0.622 (0.414–0.784)    | 0.879 (0.782–0.930) | 0.872 (0.792–0.930) |
| Edited       | 0.617 (0.431–0.773)    | 0.864 (0.778–0.925) | 0.896 (0.828–0.943) |
We also found that manual delineation of PLIC VOIs produced higher overall mean FA values than edited template VOIs, possibly because the examiners more precisely excluded subcortical gray matter when drawing the VOIs by hand. However, it should also be noted that examiners only manually delineated PLIC VOIs for around 10 image slices, whereas the template VOIs extended more superiorly and included approximately 20 image slices. The superior portion of the PLIC, where it transitions to the corona radiata, may have lower FA values due to the ‘fanning out’ of axons and crossing fibers. This could contribute to the lower mean FA values obtained from both hemispheres when examiners edited template VOIs. One of the non-expert examiners produced consistently higher mean FA values than the other examiners. This was likely due to having a conservative bias and restricting the PLIC VOIs to a smaller volume, across both hemispheres and with both methods.

There were no effects of METHOD or EXAMINER on FA asymmetry, and both methods of VOI delineation resulted in ‘almost perfect’ agreement between examiners for FA asymmetry values, despite a lesser agreement between examiners for contralesional FA values (Table 2). This indicates that FA asymmetry is a more robust measure than mean FA values for clinical application in stroke. This is because FA asymmetry detects lateralized white matter damage while compensating for examiner effects and differing methodologies, and normalizes between-subject differences in overall white matter integrity. Furthermore, the FA asymmetry measures produced by both methods were ecologically valid, as they correlated as expected with clinical scores (Table 2) (Jang et al., 2005; Sinear et al., 2012; Thomalla et al., 2004; Watanabe et al., 2001; Yu et al., 2008).

Contralesional mean FA did not correlate with age when PLIC VOIs were manually delineated (Table 3). This may have been due to under-sampling of the FA data, as the manual VOIs were limited to 10 transverse slices by the time required to draw them. A potential advantage of editing template PLIC VOIs was that the templates had a greater volume, and more of the FA data were included in the subsequent analyses. When PLIC VOI templates were edited as required, contralesional mean FA was negatively correlated with age, as expected.

To date, a direct comparison between edited and manual methods of VOI-based analysis in the PLIC has not been conducted. Previous inter-examiner reliability comparisons have mainly used the tractography approach, and been performed either with healthy subjects or patients with other neurological conditions such as head injury or amyotrophic lateral sclerosis (Borich et al., 2012; Hong et al., 2008; Ozturk et al., 2008; Qiu et al., 2011; Tang et al., 2010; Wakana et al., 2007; Zhang et al., 2008). One study that did investigate inter-examiner reliability using the manual method in chronic well-recovered stroke patients reported inter-examiner reliability values of ICC(3,1) = 0.37 for the contralesional side and ICC(3,1) = 0.72 for the ipsilesional side (Borich et al., 2012). Our study sample size is relatively large and employed more examiners compared to other related reliability studies in stroke patients (Borich et al., 2012; Qiu et al., 2011; Tang et al., 2010). The participant group was more heterogeneous for age, functional impairment and lesion location. None of the participants had a history of previous stroke and the time delay between stroke onset and MRI acquisition was relatively short. Participants also had greater upper limb impairment overall, indicating worse stroke severity compared to subjects in previous studies (Borich et al., 2012; Qiu et al., 2011; Tang et al., 2010).

The heterogeneous nature of our study sample and the enrolment of 4 examiners pose a more realistic challenge to the reliability and validity of each method in a clinical setting. We found that editing registered template PLIC VOIs as required allowed more of the PLIC to be included in analyses and improved the spatial similarity of VOIs between examiners, while avoiding examiner effects on subsequent FA measures and improving their ecological validity. We propose that editing registered template PLIC VOIs as required is feasible in a busy clinical environment, and may allow the use of FA parameters for prognoses at the sub-acute stage for post-stroke recovery of motor function.
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