ON-LINE APPENDIX: SUPPLEMENTAL MATERIALS AND METHODS

Assessment of Microstructural Changes within the Penumbra

MR Imaging Data Acquisition and Assessment of Infarction Volume. MR imaging data were acquired on a 3T Philips scanner (Achieva; Philips Healthcare, Best, the Netherlands) with standard 8-channel head coils by using consistent sequences and parameter settings. DTI was acquired by using a single-shot, spin-echo, echo-planar imaging sequence, resulting in 1 non-DWI ($b = 0$ s/mm^2) and 15 DWIs ($b = 800$ s/mm^2, 15 noncolinear gradient directions), covering the whole brain with the following parameters: TE = 55 ms, flip angle = 90°, field of view = 224 × 224 × 146 mm, 73 transverse slices, section thickness = 2 mm and a 0-mm interslice gap, voxel size = 2 × 2 × 2 mm. A whole-head, high-resolution 3D gradient-echo T1WI was acquired by using the following parameters: TE = 4 ms, TR = 9 ms, flip angle = 8°, field of view = 240 × 252 × 200.25 mm, 267 sagittal slices, section thickness =1 mm and 0-mm interslice gap, voxel size = 1 × 1 × 1 mm.

The infarction volume was assessed from DTI data within the acute poststroke phase. The area of infarction was segmented by using semiautomatic segmentation software (ITK-SNAP, www.itksnap.org[1]), with subsequent quantitative analysis. On consideration of the reconstructed trace and ADC maps, a neuroradiologist (M.T.B.) with 3 years of experience acquired the entire infarcted lesion by thresholding and manual adaptation for each patient. Subsequently, lesion volumes were extracted. Infarction growth was defined as the subtraction of lesion volume and admission infarct core, which was estimated from admission CT perfusion imaging (for details see the section in the text: Materials and Methods: Assessment of the Penumbra in Admission CT Imaging).

DTI and 3D T1 Data Processing. Diffusion data were processed by using the FMRIB Diffusion Toolbox (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT). Eddy-current distortion and head motion were corrected by linear registration of all DWIs to the first b = 0 volume. Brain-tissue extraction was performed by removing the skull and nonbrain tissue by using the FSL Brain Extraction Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET).[2] The tensor model was fitted with FSL Fitting Diffusion Tensors (https://users.fmrib.ox.ac.uk/~behrens/fdt_docs/fdt_dtifit.html) to obtain images of MD. The FMRIB Automated Segmentation Tool (FAST; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fast) was applied to segment the native 3D T1 images into different tissue types (gray and white matter, CSF) and to correct for spatial intensity variations.[3] Each image was visually checked by a neuroradiologist (M.T.B.) with 3 years of experience to identify data corrupted by artifacts.

Co-Registration of CT and MR Imaging, and Quantitative MD Analyses. After using FSL BET for brain extraction of the anatomic CT perfusion images,[2] a 2-step transformation procedure was used to register the individual CT image to the diffusion image. In the first step, linear (affine) transformation was performed between the CT image and brain extracted, and a bias-corrected native T1 image of the same patient by using the FMRIB Linear Image Registration Tool (FLIRT; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT[4,5]) with 12 *df* and the correlation ratio cost function. In the second step, the same procedure was applied to register the brain extracted b = 0 image (diffusion space) of each subject to the corresponding native T1 image. The individual diffusion-to-native and native-to-CT linear transformation matrices were combined, which resulted in individual diffusion-to-CT transformations and their corresponding inverses.

These warp fields were then applied to the individual hypoperfusion mask in CT space, extracted from CT perfusion images by the use of RAPID, to align them into each subject's individual diffusion space with the implementation of nearest neighbor interpolation, followed by a visual check by a neuroradiologist (M.T.B.) with 3 years of experience. In the intermediate step of T1 space, information of FAST segmentation was implemented to identify and map only the gray matter of the hypoperfusion masks. Previous segmented ischemic infarction maps (see section MR Imaging Data Acquisition and Assessment of Infarction Volume) were then subtracted from the hypoperfusion mask to get a mask (penumbral mask) that contains gray matter of the salvaged penumbral tissue, which, in the end, did not show visible infarction. MD values of this penumbral mask were extracted and averaged by using Matlab-based (MathWorks, Natick, Massachusetts) in-house software.

To find intra-individual gray matter alterations, MD values of the corresponding voxels in the contralateral, healthy, nonaffected side (H) were assessed and compared with MD values of the infarcted side (I). MD index was calculated by using the following formula: $\text{MD index} = (MD_I - MD_H)/(MD_I + MD_H)$. This kind of asymmetry index was previously used in stroke studies, mainly structural imaging studies, and was an accessible predictor variable.[6-8]

The assessment of contralateral voxels was achieved by swapping the penumbral mask (without applied FAST, gray matter identification was made for the contralateral tissue separately within this procedure) into the contralateral hemisphere. For this purpose, penumbral masks must be aligned to the Montreal Neurological Institute (MNI) space by using diffusion-to-standard space transformations and their corresponding inverses. They were used to warp penumbral masks into the MNI space, then swap them to the contralateral hemisphere (by using FSL-related tools) and align them back (over a step of gray matter identification [by using FAST] in individual T1 space) to diffusion space in which MD-value extraction was performed.

For registering T1 images to the MNI ICBM (International Consortium of Brain Mapping) 152 nonlinear (6th generation) symmetric standard-space T1-weighted average structural template image,[9] a 2-step transformation procedure was applied: in the first step, linear (affine) transformation was performed by using FLIRT, followed by the second step, which used the generated output to perform nonlinear registration with FMRIB's Nonlinear Image Registration Tool (FNIRT; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT). The output of this transformation procedure was an individual native-to-standard (MNI space) nonlinear warp field. The above-mentioned individual diffusion-to-native transformation matrix was combined with the just-described native-to-standard nonlinear transformation matrix, which resulted in diffusion-to-standard space transformations, which were used for alignment.

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