Experimental Assessment of Infarct Lesion Growth in Mice using Time-Resolved T2* MR Image Sequences

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Abstract. Cerebral ischemic stroke is a major reason for death in Germany and worldwide. Although lots of research has been done on long-time (3 hrs – 27 days) stroke growth, not much is known about the acute stroke growth. In this paper a method for the image based analysis and visualization of short-time infarct growth in murine model based on time resolved T2* MR image sequences is presented. After manual definition of initial healthy and infarct volumes the corresponding histograms are extracted and used for classification of the stroke tissues for every T2* image sequence. After automatic classification computed thresholds can be used to 3D visualize stroke growth dynamically and analyze the infarct size over time. The method proposed was validated by medical experts in terms of visual comparison to histological images of the brains after stroke. The medical experts reported that the accuracy of the projected infarct size is sufficient for further analysis steps of infarct growth. In summary the method proposed can help to understand the evolution of strokes and may help to get new insights in this disease and improve the therapy for stroke patients in future.

1 Introduction

Cerebral ischemic stroke is a major reason for death in Germany and worldwide. The ischemic stroke is caused by undersupply of blood and oxygen to the brain resulting from blockage of an artery due to blood clots or pieces of fatty deposits. It is characterized by insufficient metabolic circulation as well as reduction of cerebral blood flow and high oxygen extraction fraction. A detailed understanding of cerebral stroke evolution is clinically important for the diagnosis and treatment of patients [1]. The number of publications dealing with stroke growth in human as well as in animal models is high. In clinical practice several MR modalities, which reflect histopathologic changes in the brain tissue, are used including diffusion weighted imaging (DWI), apparent diffusion coefficient

(ADC) and T2-weighted measurements. Due to transportation time to hospitals, image sequences from humans directly after an occurrence of an occlusion are not available. Due to this fact most research focuses on the question how the stroke area grows over long time. Lansberg et al. [2] for example investigated time periods ranging from 19 hours to 27 days. The focus of this study is to investigate whether signal changes of T2* weighted MR images exhibit useful variations in the ischemic hemisphere immediately after vascular occlusion, such that the acute stroke growth can be analyzed. In this study a murine model has been utilized for short time infarct growth analysis. For the quantitative analysis of stroke growth a segmentation of the corresponding volume is required, whereas manual definitions of the infarct region have been incorporated in most studies (e.g. [3]). This manual definition is very time consuming and especially because of the high number of T2* image sequences acquired in this study such a procedure was not applicable. For this reason a semi-automatic method for quantitative and qualitative acute stroke evolution analysis based on T2* weighted MR image sequences was developed and is presented in the following.

2 Material and Methods

2.1 Stroke Model

Cerebral ischemia stroke was induced in male adult mice (n=18, 10-18 weeks) following [4]. Briefly, the animals were anesthetized intraperitoneally by ketamine (115,3 mg/kg, concentration of 25 mg/ml) and xylazine (4 mg/kg, concentration of 20 mg/ml) for all procedures. Temporary middle cerebral artery occlusion (tMCAO) was induced by the intraluminal filament model by inserting a nylon filament (6-0) with a 24 μm rounded tip via the external carotide artery into the internal carotide artery. The common carotide artery was ligated before. The filament was gently forwarded 9-10 mm measured from the bifurcation. The animal was immediately placed in a u-shaped animal rail and MR imaging was performed. If needed, animals were re-anesthetized intraperitoneally with half the above dose. After 30 or 60 minutes, for reperfusion, animals were removed from the scanner and the filament retracted 7-9 mm under visual inspection. Mice were replaced into the scanner and measurements continued. All animal experiments described were approved by the local ethics committee.

2.2 MR Image Sequences

Misery perfusion and acute ischemia affect magnetic resonance signal intensities due to changes of the blood oxygenation level [5]. To measure a net increase in local tissue deoxyhemoglobin the blood oxygenation level-dependent (BOLD) technique can be used. Especially T2* weighted imaging as an indicator of changes in the local concentration of deoxyhemoglobin based on the BOLD effect became important for the analysis of an acute cerebral ischemic infarction in the past. For this study among others dynamic T2* weighted imaging was performed

using a 3.0 T Intera Philips MR device. The resulting dynamic T2* weighted image sequences were acquired using a repetition time of 1 second, an echo time of 9.21 ms, a flip angle of 25°, image in-plane image resolution of 0.23 mm × 0.23 mm and a slice thickness 0.3 mm. Using this setup 25 dynamic 3D images were acquired per mouse with a temporal resolution of approx. 2 min.

2.3 Stroke Analysis and Visualization

The first step of the semi-automatic method for short-time stroke growth analysis is the preprocessing of the available data. Due to saturation effects a linear increase of the intensity values over time can be observed. For reduction of this, intensity equalization using a histogram matching technique is performed first. Then the first time point of the 4D T2* weighted image sequence is defined as the baseline in which the core of the stroke is already represented by reduced intensities. In order to allow a precise analysis of stroke growth the core infarct volume of interest (VOI) is defined manually by medical experts. In doing so the user can manually select points in the orthogonal views which are connected using Bezier curves. Besides the infarct volume a healthy VOI is also required for the following analysis and can be defined in the same manner Fig. 1(a). These VOIs are then used to compute the corresponding histograms for the defined infarct and healthy volumes in the baseline. In general it can be expected that the infarct volume is represented by lower intensities than a healthy volume in T2* image sequences. Based on this assumption the following classification of the temporal stroke growth can be performed. For classification the histograms of the extracted VOIs are normalized Fig. 1(b, c). The infarct histogram is subtracted from the healthy histogram and for noise reduction the resulting representation is B-Spline approximated Fig. 1(d). This representation assigns positive values to voxels which are more likely to be part of the infarct volume and negative values to voxels part of the healthy brain tissues. Based on this representation the positive and the negative part are both divided into 8 bins of equal areas under the graph such that each intensity value can be assigned to

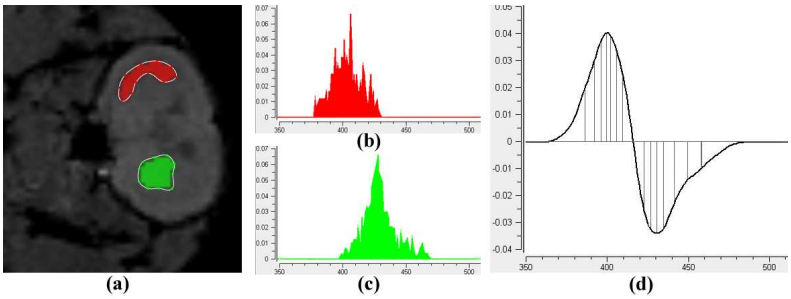


Fig. 1. Slice from a T2 MR image sequence with healthy (green) and infarct (red) VOI (a), corresponding histograms (b-c) and combined histogram representation divided into 16 bins (d).

one of the resulting 16 bins Fig. 1(d). This representation is then used for the analysis of stroke growth. For this the brain tissue is segmented in every available image. Normally the brain can be easily separated in T2* image sequences, such that a simple thresholding and a following largest connected component analysis leads to sufficient results. Based on the lower and upper thresholds of the computed bins, each voxel inside the brain segmentation is assigned to one of the computed bins Fig. 2. After this procedure is carried out for every time point the user can interactively chose which bins are supposed to be used for the dynamic 3D visualization of the stroke growth Fig. 3. The possibility of choosing different bins allows the visualization of different infarct areas such as the core or the penumbra. For visualization purposes the user defined infarct VOI is used to extract voxels part of the chosen bins for every time point. These voxels are then used for a seeded region growing in the bin-dataset, such that voxels outside the initial VOI can also be segmented. Finally this segmentation can be used for surface model generation using the Marching Cubes algorithm which can be displayed over time. Furthermore the extracted segmentation can be used for stroke quantification over time.

3 Results

The qualitative evaluation of the method proposed was validated by visual inspection by neuroradiologic experts. In a first step the semi-automatic computed final infarct volumes were compared to histological cuts of the corresponding brains of each dataset using different thresholds. Based on these thresholds, dynamic visualizations of all datasets were computed and inspected in regard to plausibility. In general the visualizations of the infarct growth were rated to display a feasible behavior. Especially the possibility using different bins for visualization was found to be helpful to investigate different stroke growth patterns. The visualizations were rated as helpful to get new insights into the short-time growth of ischemic strokes, especially the interactive navigation through time and 3D space, including rotation and zooming was judged as a benefit.

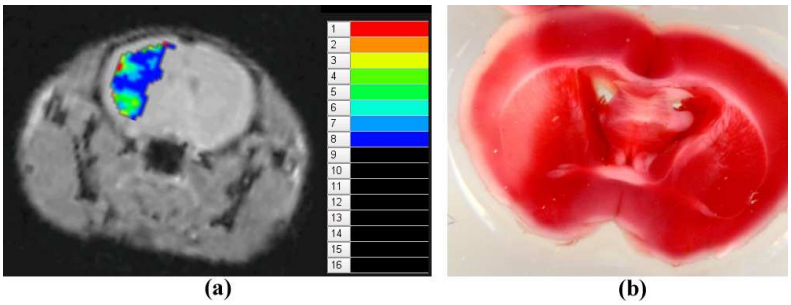
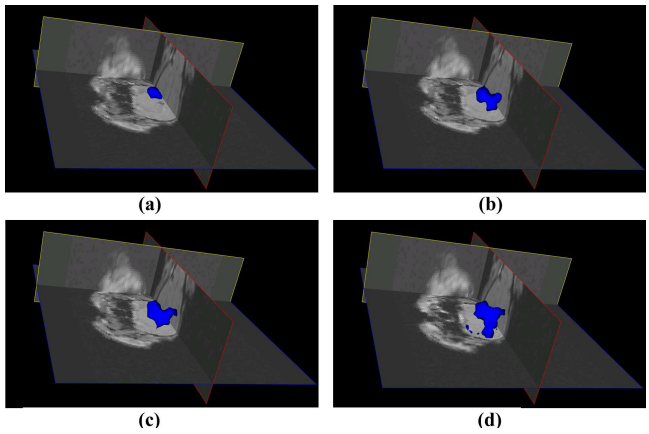


Fig. 2. Slice from a T2* MR image sequence and color-coded (bin 1-8) extracted infarct region (a) and corresponding histological slice from mouse brain (b).

Fig. 3. Selected frames of the dynamic visualization of acute stroke growth for the timepoints t = 0min (a), t = 18 min (b), t = 36 min (c) and t = 50 min (d).



4 Discussion

In this paper a method for visualization and quantitative analysis of infarct growth was presented. Based on one initial healthy and infarct volume of interest the stroke can be segmented for every time point available using a histogram based classification and dynamically displayed over time. It is planned to include the information of the histological images for automatic VOI definition in order to reduce the manual interaction time. At the moment the method proposed is used for a clinical trial and first medical results will be published soon. Further more post-occlusion image sequences will be included in order to quantify the tissue which is reactivated after occlusion has been removed. In summary the method presented can help to explore alterations explicitly during the first few minutes of vascular occlusion and can help to understand the evolution of strokes.

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