NeuroImage: Clinical 12 (2016) 219–226

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Diffusivity in multiple sclerosis lesions: At the cutting edge?☆

Alexander Klistorner[a,b,*], Chenyu Wang[c], Vera Fofanova[b], Michael H. Barnett[c], Con Yiannikas[d], John Parratt[d], Yuyi You[a,b], Stuart L. Graham[b]

[a]Save Sight Institute, Sydney Medical School, University of Sydney, Sydney, Australia
[b]Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia
[c]Brain and Mind Research Institute, Sydney Medical School, University of Sydney, Sydney, NSW, Australia
[d]Royal North Shore Hospital, Sydney, NSW, Australia

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 4 June 2016
Accepted 4 July 2016
Available online 05 July 2016

ABSTRACT

Background: Radial Diffusivity (RD) has been suggested as a promising biomarker associated with the level of myelination in MS lesions. However, the level of RD within the lesion is affected not only by loss of myelin sheaths, but also by the degree of tissue destruction. This may lead to exaggeration of diffusivity measures, potentially masking the effect of remyelination.
Objective: To test the hypothesis that the T2 hyperintense lesion edge that extends beyond the T1 hypointense lesion core is less affected by tissue loss, and therefore a more appropriate target for imaging biomarker development targeting de- and re-myelination.
Method: Pre- and post-gadolinium (Gd) enhanced T1, T2 and DTI images were acquired from 75 consecutive RRMS patients. The optic radiation (OR) was identified in individual patients using a template-based method. T2 lesions were segmented into T1-hypointense and T1-isointense areas and lesion masks intersected with the OR. Average Radial, Axial and Mean diffusivity (RD, AD and MD) and fractional anisotropy (FA) were calculated for lesions of the entire brain and the OR. In addition, Gd enhancing lesions were excluded from the analysis.
Results: 86% of chronic T2 lesions demonstrated hypointense areas on T1-weighted images, which typically occupied the central part of each T2 lesion, taking about 40% of lesional volume. The T1-isointense component of the T2 lesion was most commonly seen as a peripheral ring of relatively constant thickness ("T2-rim"). While changes of diffusivity between adjacent normal appearing white matter and the "T2-rim" demonstrated a disproportionally high elevation of RD compare to AD, the increase of water diffusion was largely isointense between the "T2-rim" and T1-hypointense parts of the lesion.
Conclusion: Distinct patterns of diffusivity within the central and peripheral components of MS lesions suggest that axonal loss dominates in the T1 hypointense core. The effects of de/remyelination may be more readily detected in the "T2-rim", where there is relative preservation of structural integrity. Identifying and separating those patterns has an important implication for clinical trials of both neuroprotective and, in particular, remyelinating agents.

1. Introduction

Recent interest in the development of remyelinating therapies has increased demand for reliable in vivo surrogate markers of remyelination. Quantification of the diffusion characteristics of brain tissue, in particular Radial Diffusivity, has been suggested as a promising imaging biomarker associated with the level of tissue myelination. Experimental models of demyelination have demonstrated a close correlation between degree of myelin loss and alterations in RD (Song et al., 2005; Janve et al., 2013). In post-mortem studies of human MS brains, elevation of RD was topographically linked to areas of histologically identified demyelination (Schmierer et al., 2007; Schmierer et al., 2008; Wang et al., 2015). A close relationship between increase in RD and electrophysiological measures of demyelination was also reported in patients with MS (Alshowaeir et al., 2014). However, some recent studies have failed to demonstrate an unequivocal relationship between increased RD and the degree of demyelination, suggesting that this measure is not pathologically specific (Klawiter et al., 2012).

Currently, conventional MRI is the gold standard to identify focal inflammatory demyelination in MS. Typically, acute T2 lesions comprise an area of demyelination and surrounding edema. Resolution of acute inflammation and edema are probably responsible for reduction in lesion size, with a permanent residual lesion that includes demyelinated

☆ Supported by grant from National Multiple Sclerosis Society (NMSS), Novartis Save Neuron Grant, Sydney Eye Hospital foundation grant YY is National Health and Medical Research Council (NHMRC) fellow.
* Corresponding author at: Save Sight Institute, University of Sydney, 8 Macquarie St. Sydney, NSW 2000, Australia.
E-mail address: sasha.klistorner@sydney.edu.au (A. Klistorner).

http://dx.doi.org/10.1016/j.nicl.2016.07.003

(and partially remyelinated) axons (Schmierer et al., 2009) and areas of expanded extracellular space. The widening of extracellular space is believed to be caused by tissue destruction and may occupy up to 87% of the lesion volume (Barnes et al., 1991; Miller, 2008). The majority of chronic T2 lesions are also seen as hypointense areas on T1-weighted images, although typically the change of the signal intensity on the T1-weighted image occurs only in part of the T2 lesion (Barkhof and van Walderveen, 1999). The level of T1 hypointensity varies significantly from slightly hypointense in comparison to surrounding NAWM to approaching the intensity of CSF, reflecting a variable degree of widening of the extracellular space (Loevner et al., 1995; Van Waesberghe et al., 1998; Miller, 2008).

The extent of lesional tissue loss is also closely linked to DTI measures, since expansion of extra-cellular space dramatically increases isotropic diffusion of water molecules (Rovaris et al., 2005). Therefore, it is highly likely that the level of RD within the lesion is affected not only by loss of myelin sheath, but also by the level of tissue destruction. Tissue destruction may therefore 'exaggerate' both RD and AD (Wang et al., 2011), masking the potential effect of remyelination on diffusivity measures.

The purpose of this study was to examine diffusivity indices in T2 and T1 lesions of MS patients. Since T1 changes, which are closely relate to loss of tissue matrix, constitute only part of a T2 lesion, we hypothesized that the T2 hyperintense lesion edge that extends beyond the T1 hypointense lesion core (which we called "T2-rim" area) may be less affected by tissue loss, and therefore be a better target for studying the effects of de/remyelination.

The specificity of altered diffusion for pathologic changes is limited by the wide spectrum of normal anisotropy indices in the brain (Bammer et al., 2000). We studied lesions in the optic radiations, highly organized fibre tracts that are a frequent site of MS pathology, to facilitate accurate measurement of relative diffusivity change along axonal bundles (Mädler et al., 2008). In addition, internal structure of the OR does not contain a significant number of crossing fibers, which can potentially (and sometimes paradoxically) alter diffusivity (Yeatman et al., 2012; Winston, 2012). This point is especially pertinent considering the issues that surround misalignment between corresponding eigenvectors with the underlying tissue structures (Wheeler-Kingshott and Cercignani, 2009).

2. Material and methods

The study was approved by University of Sydney and Macquarie University Human Research Ethics Committees. All procedures followed the tenets of the Declaration of Helsinki and written informed consent was obtained from all participants.

2.1. Subjects

Seventy-five consecutive patients with Relapsing-Remitting MS (RRMS) and no history of clinical optic neuritis (ON) in at least one eye were enrolled. RRMS was defined according to standard criteria (Polman et al., 2011). A history of ON was based on the patient's clinical notes and the absence of previous symptoms. Patients with any other systemic or ocular disease, in particular those that could potentially affect our measurement parameters were excluded.

2.2. MRI protocol

The following sequences were acquired using a 3T GE Discovery MR750 scanner (GE Medical Systems, Milwaukee, WI):

1. Pre- and postcontrast (gadolinium) Sagittal 3D T1: GE BRAVO sequence, FOV 256 mm, Slice thickness 1 mm, TE 2.7 ms, TR 7.2 ms, Flip angle 12°, Pixel spacing 1 mm. Acquisition Matrix (Freq. × Phase) is 256 × 256, which results in 1 mm isotropic acquisition voxel size. The reconstruction matrix is 256 × 256.

2. FLAIR CUBE; GE CUBE T2 FLAIR sequence, FOV 240 mm, Slice thickness 1.2 mm, Acquisition Matrix (Freq. × Phase) 256 × 244, TE 163 ms, TR 8000 ms, Flip angle 90°, Pixel spacing 0.47 mm. The reconstruction matrix is 512 × 512.

3. Whole brain 64-directions diffusion weighted imaging with 2 mm isotropic acquisition matrix (TR/TE = 8325/86 ms, b = 1000 s/mm^2, number of b0s = 2).

2.3. Reconstruction of individual optic radiations

Individual optic radiation masks was reconstructed from in-house optic radiation template through non-linear registration as follow:

1. 3DT1 images were lesion-inpainted (FSL) using T2 lesion masks,
2. individual brain masks were derived using Brain Extraction Tool from FSL with manual quality control,
3. deformation maps were obtained from non-linear registration between individual brain and ICBM 2009a standard brain template (Fonov et al., 2011),
4. in-house optic radiation template was mapped into individual patient's space through the deformation map.

2.4. Lesion identification and analysis

Whole brain T2 lesions were identified on the co-registered T2 FLAIR images and segmented semi-automatically using ITK-SNAP 3 software (http://www.itksnap.org) by a trained analyst. To minimize partial volume effect, only T2 lesions with a volume larger than 100 mm^3 were evaluated.

T1 lesions were identified on pre-contrast 3D-T1-weighted images. They were defined as regions with low signal intensity relative to the surrounding white matter and corresponded with an area of high signal intensity on T2. The lesion (hypo-) intensity was not quantified. T2 lesions demonstrating Gd-enhancement were excluded from analysis. Similar to T2 lesions, T1 lesions were segmented semi-automatically using ITK-SNAP 3 software by a trained analyst (VF). Both T2 and T1 lesion segmentation was verified by two more persons, who have extensive experience in lesion segmentation (AK, CW). Occasional discrepancies were resolved by consensus.

Each T1 hypointense lesion typically occupied the central area of a T2 lesion, leaving the peripheral part of the T2 lesion T1-isointense. We called this part of the T2 lesion the "T2-rim" (Fig. 1). A minority of T2 lesions did not display T1 hypointensity; these lesions were typically small or had a linear, elongated shape (blue arrowheads in Fig. 1), contributing little to the total T2 lesion volume. Therefore, for the purpose of analysis, T1-hypointense and T2-rim areas of T2 lesions were evaluated separately.

Since the majority of lesions had a spherical shape, we calculated radius of T2 and T1 lesions by dividing the total lesion load by the lesion number. In addition, the width of the T2-rim area was calculated as a difference between the radii of T2 and T1 lesions.

Lesion masks were then intersected with the OR to identify and measure the volume of T2, T1 and T2-rim lesions within the OR, as described in detail elsewhere (Klistorner et al., 2015).

For single lesion analysis, only well demarcated T2 lesions with no visible abnormality in the corresponding part of contralateral OR on both T2 and T1 images were selected. The region of the contralateral OR corresponding in volume and position to T2 lesion was used as a reference representing NAWM (Fig. 2).

3. Results

Seventy-five consecutive RRMS patients (age: 41.6 ± 10.1, disease duration: 4.9 ± 3.6 years, 25M/50F, EDSS score: 1.42 ± 1.38) were enrolled in the study. Patients with any other systemic or ocular diseases were excluded. All patients were relapse-free for at least 3 months