Advances in Functional Spine Neuroimaging

Feroze Mohamed, PhD[1]; Chris Conklin, PhD; Sona Saksena, PhD; Mahdi Alizadeh, MS; Devon Middleton, MS

[1]Department of Neurology, Thomas Jefferson University, Philadelphia, PA

Several imaging modalities are currently being used to obtain diagnostic information in patient with spinal cord injuries. Among them, magnetic resonance imaging, computed tomography myelography, and plain radiography are the most widely used. Magnetic resonance imaging or MRI is a non-invasive imaging method that uses magnetic fields and radio frequency (RF) waves and provides soft tissue contrast of the spinal cord and surrounding tissues within the spinal canal. On the other hand, computed tomography or CT is based on x-rays, to provide excellent bone contrast, and is the first line of diagnostic imaging performed following a traumatic injury in both adults and kids to evaluate for fractures and spinal subluxation. Subsequently, MRI is performed to evaluate for the presence of spinal cord compression, spinal cord edema and/or hemorrhage, epidural/subdural hemorrhage, prevertebral edema, and ligamentous injury. Although still not widely available, in addition to providing good structural information, MRI has evolved in the recent years to provide functional characteristics of the spinal cord. These include information such as diffusion of the water molecules within the spinal cord providing functional information of white matter based on diffusion tensor imaging (DTI), and neuronal activation sites within the gray matter of the spinal cord based on Blood oxygenation level dependant (BOLD) imaging. In our center at Jefferson we are utilizing these functional neuroimaging biomarkers to potentially help us to understand the mechanisms of spinal cord injury (SCI) as well as guide and track changes of new therapeutic procedures. In the following sections we will discuss the methodologies underlying these techniques.

DIFFUSION TENSOR IMAGING

DTI is a relatively new non-invasive MR imaging technique that quantifies the diffusion of water molecules in directions parallel and transverse to the axis of neuronal axons. Water is located intracellularly as part of the axoplasm, and extracellularly in small amount due to the dense packing of axons. Anisotropic diffusion occurs when water molecular displacement is not similar in all directions. In an axon, water molecules move further, on average, along the axonal length than across. The dense characteristics of the axon may seem to present a barrier to water diffusion perpendicular to the axon, however, several studies have ruled out the role of neurofilaments and microtubules in axonal anisotropy (Beaulieu 2002). These studies examined large diameter axons, 300 μm, where water molecules do not encounter membrane barrier. A typical diffusing water molecule travels a distance of about 5 to 11 μm within a diffusion time of 40ms. Results from these studies showed that in the absence of membrane barriers, diffusion is isotropic, even if molecules encounter neurofilaments and microtubules (Beaulieu, Allen 1994, Takahashi, Hackney et al 2002, Johansen-Berg, Behrens 2009, Beaulieu, Allen 1994). These studies eliminated the role of neurofilaments and microtubules in the observed axonal anisotropic diffusion. The other two structures believed to contribute to the restriction of water molecules movement are the axonal membrane and the myelin sheath. In the intact spinal cord, the axons and myelin sheaths in the white matter are oriented longitudinally, thus water diffusion typically occurs in this direction. In gray matter, this type of axonal organization is lacking. Thus, DTI has the ability to differentiate white and gray matter, quantify directionality of the white matter tracts, provide quantification of three-dimensional water diffusivity, track healthy nerve tissue within white matter, and assess structural damage of the cord. DTI has the potential to fill the void left by conventional MRI and International Standards for Neurologic Classification of Spinal Cord Injury (ISNCSCI) in the assessment of damage to white matter tracts within the spinal cord (Cheran, Shanmuganathan et al 2011, Mulcahey, and Samdani et al 2011). For example, DTI can reveal the precise location of the injury, which conventional MRI often fails to show. Parameters generated from DTI data may provide new metrics in the evaluation of subjects with SCI and improve accuracy in the classification of injury severity. DTI can also be used to extract different bundles of white matter tracts in the spinal cord for analyzing motor and sensory functions separately. DTI has the potential to become a reliable and valid method to evaluate and classify the neurologic consequence of SCI.

There are several diffusion parameters or indices that are extracted from these DTI measurements that reflect certain aspects of the white matter biological characteristics. The most prominent of these terms are defined below. Mean diffusivity (MD) measured in mm^2/s refers to the average displacement of the water molecules within the white matter. Radial diffusivity (referred to as RD or λT) and axial diffusivity (referred to as AD or λL) also measured in mm2/s represent the diffusivities perpendicular and parallel to the white matter tracts, respectively (LeBihan, Mangin et al 2001, Ellingson, Ulmer et al 2008). The degree of anisotropy can also be measured in spinal cord white matter. A high index of anisotropy is indicative of healthy white matter structure. Fractional anisotropy (FA) is a ratio from 0-1 that represents the degree to which diffusion is anisotropic. High FA (highly anisotropic) values indicate that diffusion is greater in one direction than in other directions whereas low FA (highly

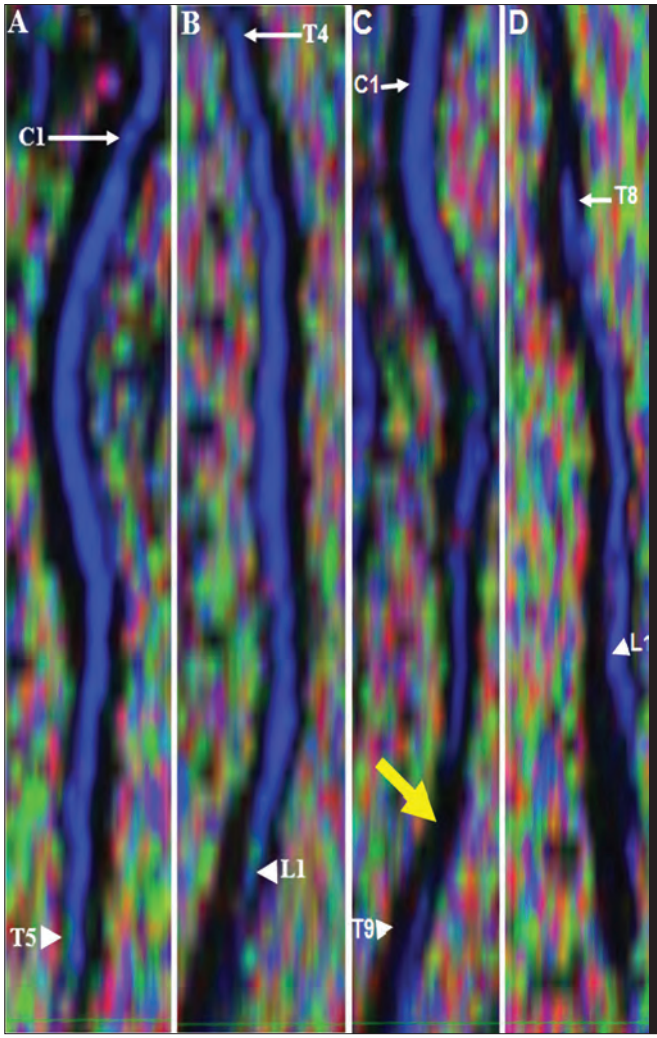

Figure 1

Sagittal reconstruction of FA color maps of two overlapping slabs of a representative normal subject (A, B) and SCI patient (C, D). Fig 1A shows the cervical and upper thoracic region (C1-arrow to T5-arrowhead) and Fig 1B shows the upper thoracic through the conus region (T4-arrow to L1-arrowhead). Fig 1C shows the cervical and middle thoracic region (C1-arrow to T9-arrowhead) and Fig 1D shows the middle thoracic through the conus region (T8-arrow to L1-arrowhead). Arrow (yellow) represents the level of injury. (Saksena, S et al, ANNR, 2016, In Press).

isotropic) values indicate diffusion is relatively equal in all directions. In the healthy spinal cord, FA values are high reflecting the movement of water molecules along longitudinal spinal tracts. In contrast, in the injured cord, FA values are low due to the breach in the longitudinal tracts allowing for diffusivity in multiple directions (Ellingson, Ulmer et al 2008, Barakat, Hunter et al 2011). There are various other DTI indices such as Diffusion Tensor Kurtosis maps and Diffusion tensor tractography images one can derive from this imaging method but their clinical significance is still under investigation.

Figure 1 shows a sagittal reconstruction of FA color maps of two overlapping slabs of a representative normal subject (A,B) and SCI patient (C,D). Fig 10A shows the cervical and upper thoracic region (C1-arrow to T5-arrowhead) and Fig 1B shows the upper thoracic through the conus region (T4-arrow to L1-arrowhead). Fig 10C shows the cervical and middle thoracic region (C1-arrow to T9-arrowhead) and Fig 2D shows the middle thoracic through the conus region (T8-arrow to L1-arrowhead). Arrow (yellow) represents the level of injury.

Figure 2. Shows a T2- and diffusion tensor tractography of a healthy subject acquired from entire cervical spinal cord. T2 weighted axial (lower left), along with reconstructed coronal (upper left) and sagittal (upper right) views and diffusion tractography of entire cervical spinal cord (lower right).

Figure 3. shows a T2- and diffusion tensor tractography of a SCI subject acquired from entire cervical spinal cord.HT2 weighted axial (lower left) along with reconstructed coronal (upper left) and sagittal (upper right) views and diffusion tractography of entire cervical spinal cord (lower right). Three dimensional tractography shows the disrupted fiber tracts of the cervical spinal cord exactly at the level of injury shown by arrow in T2 weighted image.

Figure 4. Diffusion and kurtosis maps at the mid-C5 level for a healthy adolescent. Color maps are provided for visualization and help enunciate different contrast mechanisms.

Figure 5. Diffusion and kurtosis maps of a SCI patient showing the various maps at three different locations in the cervical cord. The functional maps at the C7-T1 level show abnormalities consistent with spinal cord trauma.

CLINICAL CORRELATIONS OF DIFFUSION TENSOR IMAGING IN PATIENTS WITH SPINAL CORD INJURY

In recent studies from our Center (Mulcahey, Samdani et al 2013), correlation of DTI values with values from the ISNCSCI and MRI was examined to evaluate the strength of DTI as a clinical biomarker to evaluate spinal cord damage. The mean and standard deviation values for DTI parameters were calculated based on the measurements from DTI scans of each subject. Mean values were compared by groups using analysis of variance (ANOVA) for repeated measures. Mean values were also compared by region of the cord at the ISNCSCI motor and MRI levels with regions remote from motor and MRI level of injury. This study showed that DTI values differ not only between uninjured and injured cords but also between complete and incomplete injuries. This finding is clinically relevant as the ability to distinguish between complete and incomplete injuries using the clinical examination is difficult and when performed yields high false positives (suggesting injury is incomplete when it is actually complete). We have also shown that compared with radiologist examination of conventional T1- and T2-weighted MRI images, DTI values have shown good correlation with the clinical

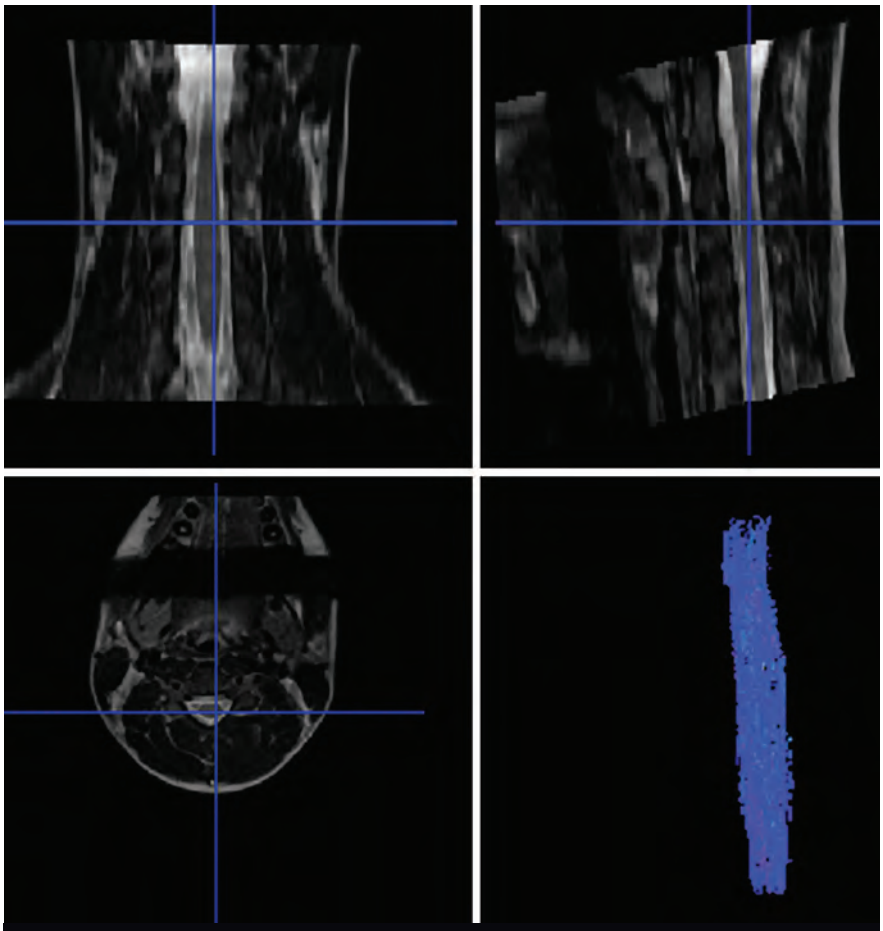

Figure 2

T2- and diffusion tensor tractography of a healthy subject acquired from entire cervical spinal cord. T2 weighted axial (lower left), along with reconstructed coronal (upper left) and sagittal (upper right) views and diffusion tractography of entire cervical spinal cord (lower right). (Alizadeh M, et al, Spinal Cord, 2016, In Press).

measures. FA had a statistically significant correlation with all clinical values with the exception of anal contraction and total pinprick. To summarize, DTI appears to be superior to conventional MRI in correlating with the clinical examination, quantifying the degree of white matter integrity, and potentially monitoring the effect of therapy in SCI.

BLOOD OXYGENATION LEVEL DEPENDANT IMAGING IMAGING

Functional MRI based on Blood Oxygenation Level Dependant Imaging (BOLD) is an indirect method of measuring neuronal activation (Ogawa, et al 1990). This method has been used extensively to study brain functions and to a lesser extent the spinal cord (Madi, Flanders et al 2001, Stroman, Nance et al 1999). Brain activity as represented by synaptic firings is associated with increased energy demands, which under normal conditions are satisfied by way of oxidative glucose metabolism. At the onset of brain activity in a particular region, local feeding arterioles dilate increasing blood flow in downstream capillaries. Although at the no-stimulus baseline all capillaries are already perfused, regional brain activity increases blood flow through the capillaries in an immediate vicinity of active neurons. The amount of oxygen brought by this influx is in excess of what is consumed through oxidative glucose metabolism. As a result, overall fraction of oxygenated hemoglobin is increased. In the configuration with bound oxygen, ferrous iron of the heme molecule changes its conformation and becomes progressively more diamagnetic (less paramagnetic) as more oxygen molecules bind. Diamagnetism of oxygenated hemoglobin (oxyhemoglobin) imparts reduced magnetic susceptibility when placed in the magnetic field of an MR scanner and thus shows an increase in MRI signal. This method is called BOLD imaging and is being used to study various brain functions as well as spinal cord activations. BOLD fMRI has been used by researchers in pediatric populations for the purpose of classifying the severity of SCI in children (Krisa L, 2013).

BOLD imaging signal however is low in spinal cord and an alternative method to observe the activation in the spinal cord directly, called Signal enhancement by extravascular water protons (SEEP) was first proposed by (Stroman, Krause et al 2001). SEEP contrast is based on changes in tissue water content that arise from the increased production of extracellular fluid and swelling of neurons and glial cells at sites of neuronal activity. Because the dominant sources of MRI signal in biological tissues are water and lipids, an increase in tissue water content is reflected by a local increase in MR signal intensity. These two functional methods are currently being used mostly under research settings however it has got great potential to be applied in the clinics to study SCI populations in the near future.

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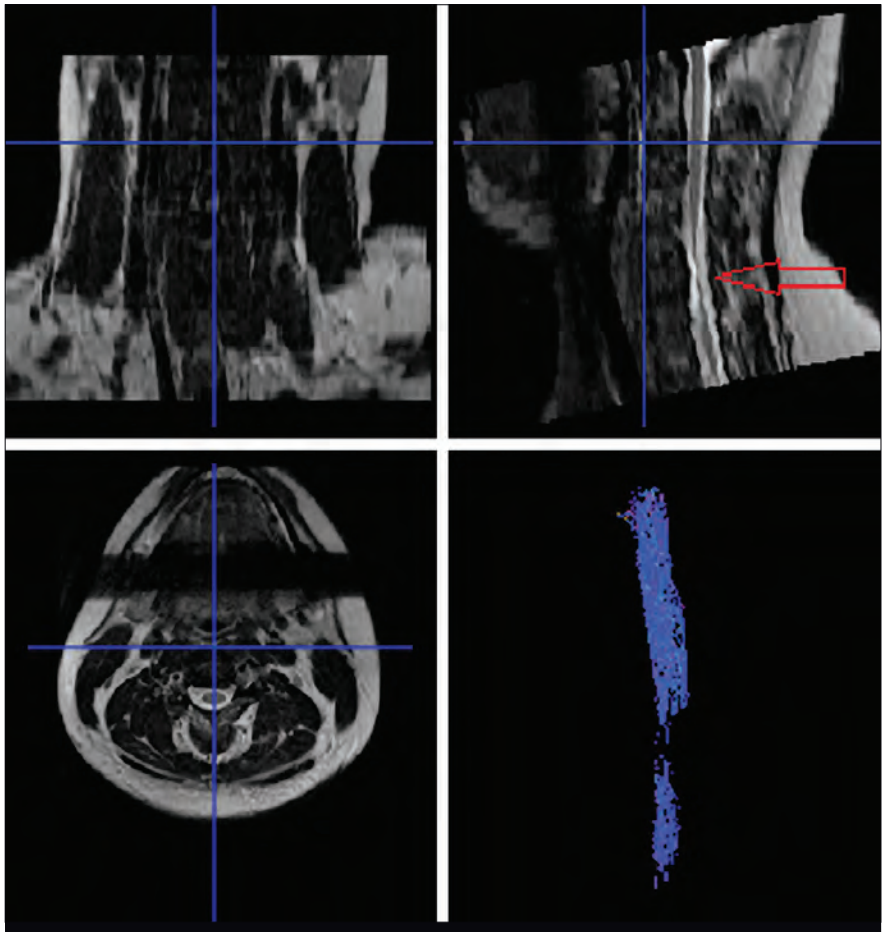

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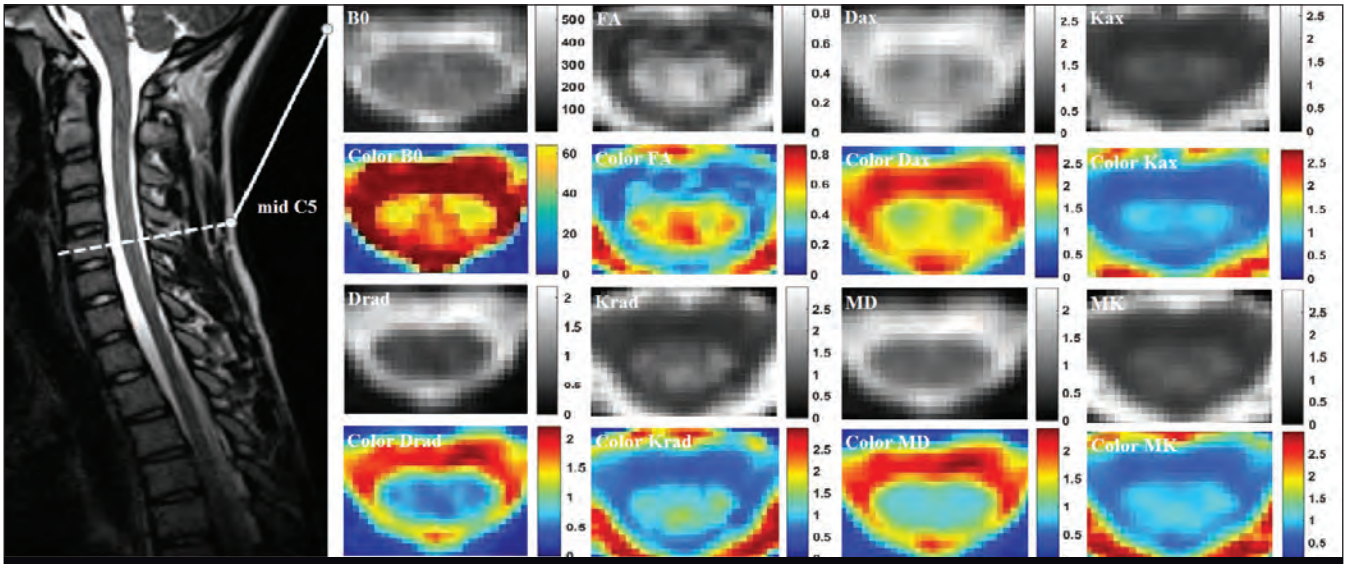

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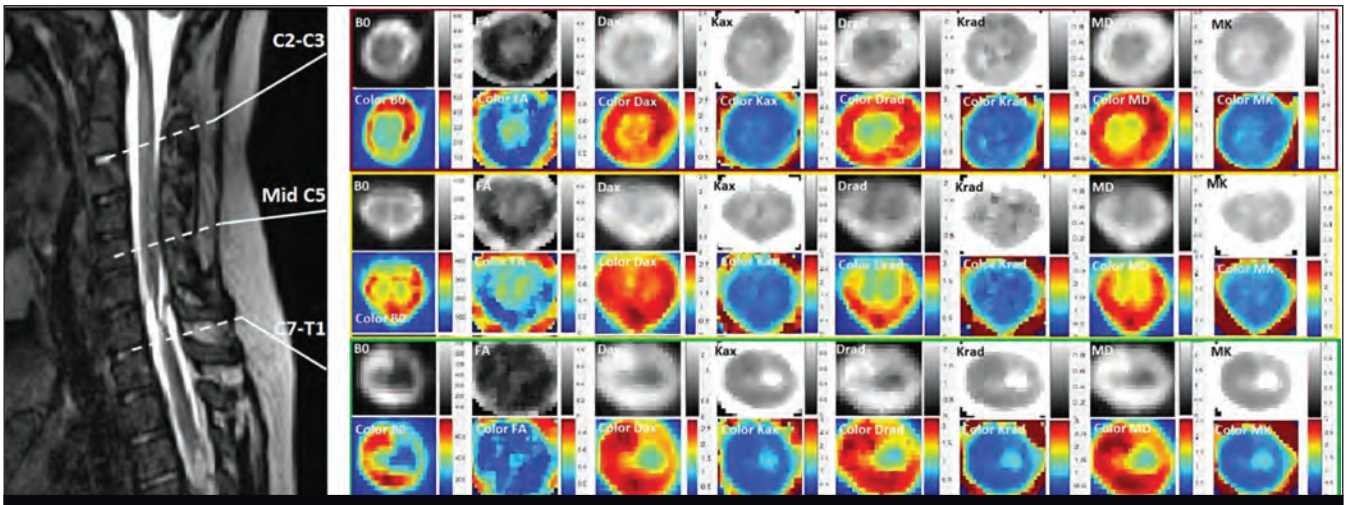

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