

Quantitative MRI Measures in SIV-Infected Macaque Brains

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Abstract

Multiple MRI modalities including Diffusion Tensor Imaging (DTI), perfusion MRI, *in vivo* MR Spectroscopy (MRS), volumetric MRI, contrast-enhanced MRI, and functional MRI have demonstrated abnormalities of the structural and functional integrity as well as neurochemical alterations of the HIV-infected central nervous system (CNS). MRI has been proposed as a robust imaging approach for the characterization of the stage of progression in HIV infection. However, the interpretation of the MRI findings of HIV patients is complicated by the fact that these clinical studies cannot readily be controlled. Simian immunodeficiency virus (SIV) infected macaques exhibit neuropathological symptoms similar to those of HIV patients, and are an important model for studying the course of CNS infection, cognitive impairment, and neuropathology of HIV disease as well as treatment efficacy. MRI of non-human primates (NHPs) is of limited benefit on most clinical scanners operating at or below 1.5 Tesla because this low field strength does not produce high-quality images of the relatively small NHP brain. Contemporary high field MRI (3T or more) for clinical use provides impressive sensitivity for magnetic resonance signal detection and is now accessible in many imaging centers and hospitals, facilitating the use of various MRI techniques in NHP studies. In this article, several high field MRI techniques and applications in macaque models of neuroAIDS are reviewed and the relation between quantitative MRI measures and blood T-cell alterations is discussed.

Keywords: HIV; AIDS; SIV; NeuroAIDS; CD4⁺; CD8⁺; Cerebral blood flow (CBF); Diffusion tensor imaging (DTI); *In vivo* MRS; Non-human primate

Introduction

HIV-associated central nervous system (CNS) disorders have been investigated widely with diffusion tensor imaging (DTI), perfusion MRI, *in vivo* Magnetic Resonance Spectroscopy (MRS), functional MRI, magnetization transfer imaging, and contrast-enhanced MRI [1-7]. These studies demonstrate that MRI is a robust non-invasive approach in studies of HIV/AIDS patients. Abnormalities of MRI measures are more frequent in demented patients than in non-demented ones. The interpretation of these MRI measures as surrogates for stage of the disease progression is complicated, however, because these clinical studies cannot readily be controlled for route of infection, viral strain, or severity of disease. Simian immunodeficiency virus (SIV) infected macaque model exhibits neuropathological symptoms similar to those of HIV+ patients, and provides a valuable platform for studying the course of infection, cognitive impairment, and neuropathological sequelae of HIV disease in AIDS vaccine development [8-16]. In addition, the macaque model allows the MRI examination to be carried out with much higher image resolution and with multiple modalities in a single session. Dedicated monkey research scanners with high-strength gradient inserts are preferred for macaque neuroimaging [17], but such high-field scanners are available in only a few research centers worldwide.

Whole-body clinical scanners are accessible in most medical centers or hospitals and most offer adequate space to accommodate any special requirements of a scan setup. Such clinical scanners have been employed in many macaque studies. However, most of them operate at or below 1.5 Tesla and thus provide a level of signal sensitivity that is suboptimal for macaque neuroimaging studies. In recent years, high field clinical MRI scanners (3T or greater) has become available in many centers. The increased field strength provides significantly improved signal sensitivity. The advanced MRI technologies facilitate nonhuman primate (NHP) neuroimaging investigation, resulting in

more informative neuroanatomical studies which provide new insights into the brain's structure and function.

One of the hallmarks of the progression of AIDS is the extensive depletion of CD4⁺ T cell subsets, especially in untreated HIV patients [18]. A massive loss of memory-phenotype CD4⁺ T cells was observed in the intestine in early stages of infection [18,19]. CD4⁺ T cell count indicates the stage of HIV disease and is the most significant predictor of the disease progression. CD8⁺ T-cells provide a major immunological defense against HIV infection and their proliferation is driven by the virus load and associated with the state of inflammation in HIV infection [20-22], and neurological dysfunctions is correlated with the expansion of CD8⁺ T cells in the SIV infected brain [21]. Also, the CD4/CD8 ratio has been proposed as a potential biomarker for the stage of HIV infection [23]. A significant correlation between the MRI measures in CBF and DTI and the blood T-cell alterations has been demonstrated in a longitudinal study of SIV-infected macaques [24].

In the present article, the use of several MRI techniques in SIV-infected macaque neuroimaging at high field is reviewed and the relationship between these quantitative imaging measures and the CD4⁺ and CD8⁺ alterations in SIV Infection is discussed.

Equipment and Animal Handling

MRI scanner

Current whole body clinical MRI scanners have 60-cm or larger

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bore size and provide ample space to accommodate animals and equipments for a variety of experimental settings in NHP studies. This relatively large bore size facilitates animal handling and monitoring. Animals can be placed in either a supine position, or be placed in the scanner on the abdomen, in a “sphinx” position during scanning. Some commercial knee coils (such as Siemens CP extremity) can fit varying macaque head sizes very well for general imaging and MRS scans. Custom-built coils are also often employed to provide optimal performance for specialized studies. Because the MRI signal to noise ratio (SNR) increases with the magnet strength, high field or even ultra-high field MRI scanners are preferred for acquisition of the best possible images.

The average macaque brain volume is about 100 cm³, less than one-tenth that of a human brain. Since most clinical MRI pulse sequences and protocols are designed and hardcoded expressly for human brain imaging purposes, they must be reprogrammed and optimized to accommodate the smaller FOV and higher spatial resolution needed in macaque brain imaging.

Behavior tests and blood tests for T-cell counts

The neurological, behavioral, and cognitive similarity of the macaques to humans [25] can be exploited to evaluate possible cognitive sequelae to SIV infection. Toward this end, computer-based cognitive behavioral tests can be used to characterize the change of cognitive function in monkeys. Among the tests that can be performed are cued and uncued attention [26,27], Delayed Non-Matching-to-Sample (DNMS) (both acquisition and memory performance with delays) [28,29], Delayed Recognition Span-spatial condition (DRST-spatial), and Spatial Reversal (SR) [30,31]. These tests are recognized measures of attention [26,27] and memory [30,31].

In order to monitor disease progression and the correlation with behavior and MRI measures, blood samples can be collected on the day before the scan. These can then be analyzed for counts of CD4⁺ and CD8⁺ T-cell subsets, by flow cytometry [12,32].

Animal immobilization, anesthesia, and physiology monitoring

Even if subjects are deeply anesthetized, the physiological motions can often produce motion artifacts on MRI images. This can be prevented by the use of a head holder to immobilize the animal. The holder in use at our facility has plastic ear bars and a tooth bar, and is designed to permit the anesthetized animal to breathe freely during the scan.

Also, the head restraint is able to provide space for the introduction of an endotracheal tube for the administration of isoflurane, an inhaled agent that produces rapid induction of and recovery from anesthesia. Monkeys are usually kept at ~1% isoflurane during scanning. Physiological parameters such as Et-CO₂, inhaled CO₂, O₂ saturation, blood pressure, heart rate, respiration rate, and body temperature, must be monitored continuously and maintained in normal ranges during the entire scanning session.

T₁ and T₂ Weighted Structural MRI

T₁ and T₂ weighted MRI provides excellent image quality and T₂ tissue contrast for structure segmentation to examine possible volumetric abnormalities of various brain structures. Cerebral atrophy is often observed in HIV+ patients with neurological symptoms [4,7,33,34]. An

inverse correlation between ventricular size and neuropsychological function was found in previous CT study of AIDS patients [35].

In macaque models, such structural volume changes can be accessed before and after SIV inoculation from T₁ and T₂ weighted structural images. High resolution T₁ and T₂ weighted images of macaques are usually acquired with the 3D MP-Rage gradient-echo sequence and the fast spin-echo sequence respectively. The structural volumes can be estimated by manual tracing or by automatic image segmentation via FSL (www.fmrib.ox.ac.uk) for voxel-based morphometry (VBM) analysis or other atlas-guided specific segmentation software. Also, the standardized planimetry to measure the ventricle-brain ratio (VBR) and the bifrontal (BFR) and bicaudate (BCR) ratios can be used for estimation of cerebral atrophy [33,34]. Briefly, The VBR is defined as the ratio of the area of the lateral ventricles over the whole brain in the slice where the brain perimeter is maximal. The BCR is the ratio of the minimum inter-caudate distance over the corresponding whole brain width in the same slice. The BFR is the ratio of the distance between the lateral tips of the frontal horns over the corresponding whole brain width in the same slice.

Li et al. used this method to estimate the cerebral atrophy of the SIV-infected macaques [24]. No significant difference between any two different time points was observed during the study period, in agreement with those seen in asymptomatic HIV-1-infected patients [4,36]. However, the BFR, an index of cerebral atrophy, increased progressively with CD4⁺ depletion as indicated by a significant correlation with CD4⁺ T cell count during infection (Figure 1a). This finding is consistent with the result in HIV patients in which the frontopolar cortical thinning is significantly associated with lower CD4⁺ counts [37]. This result suggests that the cerebral atrophy may be still occurring during the asymptomatic stage, even though no obvious volume changes are observed after the SIV infection in comparison with that of the pre-inoculation baseline.

DTI

DTI measures the magnitude and directionality of tissue water mobility and provides a non-invasive approach to access the microstructural features of the brain white matter tissue [38,39]. However, the DTI data acquisition is very vulnerable to motion, echo time, and field inhomogeneity (susceptibility artifacts). High magnetic field provides the requisite high SNR, but produces longer T₁ and shortening of T₂ and T₂*. Therefore, strong gradients are required to achieve acceptable echo time (TE). In comparison with the gradient strength of up to 400mT/m in animal research scanners, the gradient insert in a clinical scanner typically provides only 40 mT/m per axis. Thus, the echo time in the single-shot EPI sequence can become too long and cause severe signal drop and image distortion in high-resolution DTI of macaque brains. However, since the animal is usually anesthetized and immobilized during scanning, the multi-shot EPI pulse sequence can be utilized to reduce the echo time significantly in the high resolution DTI data acquisition of macaque brains.

DTI has been extensively employed for studying CNS anatomy and pathology in AIDS and HIV patients [40-45]. Fractional Anisotropy(FA) is more sensitive than mean diffusivity (MD) and decreases significantly in regions such as splenium, genu, internal capsule, frontal lobes, parietal lobes, temporal lobes, and occipital lobes in both symptomatic and asymptomatic HIV patients [41,44,46-50].

In comparison with the numerous DTI studies in HIV patients

over a decade, DTI was just explored recently in one study of SIV macaques [24], in which, a two-segment double spin-echo EPI sequence was used for DTI data acquisition at high spatial resolution (voxel size=1.5×1.5×1.5 mm³). The DTI results of SIV macaques indicate that the whole brain FA is reduced significantly after SIV infection, in agreement with the previous results in HIV patients. In addition, longitudinal FA changes of the whole brain, splenium, genu, and frontal white matter in SIV macaques, are correlated significantly with the CD4⁺ T cell counts and/or CD4:CD8 ratio during infection, as illustrated also in figure 1b, 1c and 1d, suggesting that the longitudinal changes in FA are associated with the immune dysfunction during the acute and chronic SIV infection. Also, these results suggest that DTI is a robust and sensitive technique to evaluate neuroanatomical changes in both HIV+ patients and SIV macaques.

Perfusion MRI

Cerebral Blood Flow (CBF) can be measured with several neuroimaging techniques including PET, SPECT, and MRI. Of these, Arterial Spin-labeling (ASL) in the context of MRI uses endogenous arterial blood water as a freely diffusible tracer and is a non-invasive approach for the quantitative measurement of CBF [51-53]. ASL with a separate labeling coil can achieve high SNR CBF maps of macaques

with reduced RF exposure but requires additional hardware [54]. In contrast, the amplitude-modulated continuous ASL (CASL) technique does not need additional hardware setting and can be readily used for CBF data acquisition of humans [55]. The CASL technique has been explored and optimized for CBF mapping of SIV macaques [24]. The resting CBF maps of SIV macaques with the CASL perfusion measurement at high spatial resolution (voxel size=1.5×1.5×1.5 mm³) have been demonstrated.

CBF has been utilized sparsely in HIV/AIDS researches. Abnormal CBF has been observed in a few studies of HIV patients and proposed as a noninvasive biomarker for HIV-associated CNS damage, perhaps with the potential for classifying or predicting the degree of neurocognitive impairment [56-58]. The relation between regional CBF abnormality and pathological and neurological alteration remains unknown.

The progressive CBF changes during SIV infection have been explored in one recent study of SIV macaques [24]. It has been demonstrated that CBF in caudate and inferior medial parietal cortex of SIV-infected macaques was reduced significantly. Reduction of CBF in prefrontal cortex was nearly statistically significant, probably due to the small sample size. In addition, the progressive change of

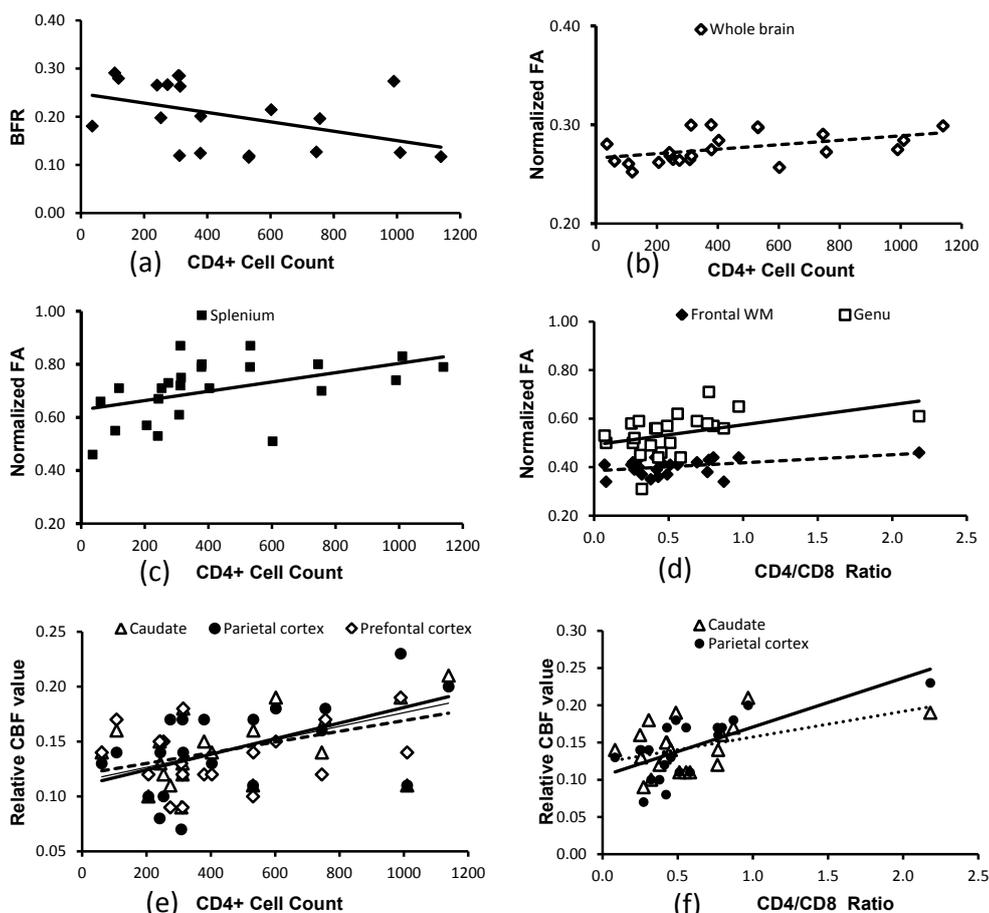


Figure 1: Multiple MRI measures correlate with CD4⁺ counts and/or CD4/CD8 ratio with P-values <0.05 confidence in a SIV-infected macaque study (Li et al. 2011). (a) brain atrophy index bifrontal ratio (BFR) correlates with CD4⁺ T cell counts (R²=0.20); (b) Fractional Anisotropy (FA) of whole brain correlates with CD4⁺ T cell counts (R²=0.21); (c) FA of splenium correlates with CD4⁺ counts (R²=0.22); (d) FA in frontal white matter and genu correlates with CD4/CD8 ratio (R²=0.17 and 0.18); (e) Cerebral Blood Flow (CBF) in caudate, parietal and prefrontal cortex correlates with CD4⁺ counts (R²=0.21, 0.29, and 0.25) (f) CBF in caudate and parietal cortex correlates with CD4/CD8 ratio (R²=0.21 and 0.50).

CBF in caudate and parietal cortex correlated significantly with CD4⁺ counts and with the CD4/CD8 ratio during infection (Figure 1e and 1f), suggesting that regional CBF in the specific structures is associated with the immune dysfunction during the SIV infection. Also, the CBF results in SIV monkeys are consistent with previous reports in HIV patients [58-63].

In vivo Proton MRS

MRS provides a non-invasive approach to measure neurochemical alterations associated with the inflammatory process during HIV infection and has been used extensively in studies of HIV or AIDS patients [59,64-75]. Metabolite abnormality was usually observed in demented and/or symptomatic HIV patients. The degree of cerebral metabolite disturbance in HIV patients is strongly associated with reduced cortical and subcortical volumes [76].

Cerebral metabolic abnormalities in SIV-infected monkeys has been examined by using localized point-resolved spectroscopy (PRESS) sequence with CHESS water suppression [77-79] or *ex vivo* MRS [80-83]. During acute SIV infection, significant reduction of NAA/Cr and Cho/Cr was observed in about 13 days and 27 days after inoculation, respectively, and the change of Cho/Cr was correlated with plasma viral load [84]. Cho/Cr in frontal lobe or NAA/Cr in basal ganglia was found correlated with plasma viral load [85]. The findings are similar to those noted in HIV-infected human brains. The NAA/Cr ratio is negatively correlated with the SIV CNS disease severity in the SIV-infected macaque model of encephalitis [78]. In particular, rapid decline in NAA/Cr ratios has been demonstrated in SIV macaques with CD8⁺ depleted [86]. The effect of the chronic morphine administration on SIV macaques has been investigated in the SIV macaque model, and the *ex vivo* MRS findings indicate the protection of chronic morphine against the neurotoxic effect of AIDS [82]. Also, the neuroprotection by oral minocycline was demonstrated in recent MRS study of the accelerated SIV macaque model [87].

Those metabolite abnormalities are not usually seen in the patients who are neurologically asymptomatic or with mild cognitive impairment [88]. In recent MRS study of SIV macaques, significant cerebral metabolite alteration was observed in a longitudinal MRS study of neurologically asymptomatic SIV macaques [89]. Also, the progressive change of NAA and glutamate/glutamine (Glx) in basal ganglia correlated with the CD8⁺ T cell percentage during the SIV infection. It is suggested that the unknown infection history and/or medication treatment may complicate the examination of *in vivo* MRS in HIV patients with no or mild cognitive impairment. In sum, *in vivo* MRS in asymptomatic macaque models may be of particular value in investigating early nervous system involvement in HIV patients with no or mild cognitive impairment.

High Field and Ultra-High Field MRI and Parallel Imaging Techniques

MRI is a non-invasive and sensitive imaging modality and being increasingly used in preclinical examination and clinical diagnosis. Two revolutionary advances in the MRI techniques emerged in recent years. These are (1) the development and application of high field and ultra-high field MRI, and (2) the advent of parallel imaging techniques.

High field (3T or more) and ultra-high field (7T or more) MRI offers increased SNR which benefits many applications using conventional and quantitative MRI methods. These include high resolution T₁ and T₂ weighted structural imaging, *in vivo* MR spectroscopy, DTI,

functional MRI, ASL-based perfusion, and susceptibility weighted imaging (SWI). The SNR of ASL-based perfusion-weighted images are further improved because of the elongated blood water T₁ at high field. However, technical challenges on magnetic field in homogeneities, RF coils, RF exposure, and increased T₁ and shortening T₂ of tissue, etc, still remain to be solved in ultra-high field MRI.

Parallel imaging technique combines multiple receiving coils in a phased array with unique imaging reconstruction algorithm to reduce the scan duration significantly. Most imaging modalities such as regular anatomical T₁ and T₂ weighted images benefit substantially from this technique in terms of improved image quality and increased scanning speed. In particular, DTI measurement is greatly improved by using the novel parallel imaging technique [90]. In our experience, a four- or eight-channel phase-array volume coil can meet general needs of macaque brain imaging. The combination of high field and parallel imaging techniques could facilitate the use of various MRI measurements of macaque brain imaging with excellent image quality and sensitivity.

Discussion and Conclusions

HIV preferentially infects the sub-cortical structures. Although it perhaps enters the CNS as early as the initial systemic infection [91], symptomatic cognitive impairment typically occurs in late stages of HIV disease when most abnormalities in quantitative MRI measures are observed. However, the specific symptoms vary from person to person, and most MRI measures are not sensitive in the examination of HIV patients with no or mild cognitive impairment, except for DTI whose robustness has been demonstrated in some studies of non-demented HIV patients [41,46,48].

SIV-infected macaques offer an ideal model for using clinical MRI scanners to characterize the CNS injury during SIV infection and the response to treatment under controlled condition. Also, a particular advantage of the macaque model is that it permits the use of high quality, multi-parameter MRI measurements in a single session to examine the CNS injury non-invasively. DTI, CBF, and MRS measurements of SIV macaques have demonstrated their robustness and efficacy to access and evaluate the CNS injury during SIV infection in previous studies. The CBF and metabolite abnormalities in basal ganglia of SIV macaques suggest basal ganglia may be more vulnerable in SIV and HIV infection.

HIV attacks and damages the human body's immune system in which the CD4⁺ T-cells play a critical role. Experimental and clinical evidence has demonstrated that CD4⁺ T cell depletion and accumulating CD8⁺ T lymphocytes are the most significant predictor of the disease severity [92]. CD4⁺ and CD8⁺ counts are typically used to access the degree of immune impairment in HIV patients. Even though clinical studies have found that CD4⁺ levels were associated with abnormalities on perfusion MRI, DTI, brain volumetric measurement in HIV patients, the relations between the CD4⁺ and/or CD8⁺ T cells and neuroimaging findings have not been conclusively identified.

The MRS results of SIV macaques indicate the cerebral metabolites are altered evidently in two weeks after SIV inoculation. Similarly, abnormal changes in FA and MD are observed also during acute SIV infection. Meanwhile, the correlation between the longitudinal DTI,

CBF, and MRS measures of CNS and altered CD4⁺ and/or CD8⁺ T cell counts during the course of SIV infection suggests that the MRI measures are sensitive to characterize the CNS injury associated with the immune dysfunction. In contrast, brain volume atrophy is not readily observed in SIV macaques and more advanced volumetric analysis may be needed to extract the subtle changes. Further investigation with these different modalities in SIV macaques may provide better understanding of the MRI findings, neurological impairment, and immune dysfunction in HIV patients.

In conclusion, high field MRI provides much improved spatial and contrast resolution and allows more accurate measurement and evaluation of macaque models of neuroAIDS. Quantitative measures in diffusion and perfusion MRI, in vivo MRS, and other modalities can be employed readily to characterize the CNS injury associated with the immune dysfunction during the course of SIV infection and relevant treatment responses. Also, with the technological advances in MRI, the methodology and application in SIV-infected macaque models are continuing to evolve and be redefined.

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