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Brain Integrity Changes underlying Cognitive and Functional Recovery Postliver Transplant Continue to Evolve Over 1 Year

Vishwadeep Ahluwalia, PhD¹, James B Wade, PhD², Melanie B White, RN¹, HoChong S Gilles, FNP¹, Douglas M Heuman, MD¹, Michael Fuchs, MD¹, Edith A Gavis, RN¹, Andrew Fagan, BS¹, Leroy R Thacker, PhD³, Richard K Sterling, MD¹, R Todd Stravitz, MD¹, Puneet Puri, MD¹, Arun J Sanyal, MD¹, Muhammad S Siddiqui, MD¹, Scott Matherly, MD¹, Velimir Luketic, MD¹, Joel Steinberg, MD², F Gerard Moeller, MD², and Jasmohan S Bajaj, MD¹

¹Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA, USA

²Psychiatry, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA, USA

³Biostatistics, Virginia Commonwealth University and McGuire VA Medical Center, Richmond, VA, USA

Abstract

Background—There is evidence of brain recovery on brain MRI early postliver transplant(LT) but the longer-term impact is unclear. Aim of this study was to determine the change in brain MRI parameters, cognition and health-related quality of life (HRQOL) between 6 and 12 months post-LT.

Methods—Listed cirrhotics underwent cognitive, HRQOL and brain MRI pre, 6 months (post-LT1) and 1-year (post-LT2) post-LT. Assessment of MRI changes between visits was performed for ammonia-associated metabolite changes using spectroscopy (MRS), white matter changes using Tract-based Spatial Statistics (TBSS) analysis on Diffusion Tensor Imaging (DTI) data and grey matter changes using Voxel-based morphometry (VBM) analysis on 3D high resolution T1-weighted images.

Results—Forty-five patients were included of which, twenty-three were tested at all visits. Cognitive and HRQOL scores improved between all visits compared to pre-LT values. This trend continued on MRS with reduced glutamine+ glutamate(Glx) and higher myoinositol(mI), Choline(Cho) between pre-LT/post-LT1 but lower degrees of improvement between post-LT1/post-LT2. On DTI, mean-diffusivity(MD), linear-diffusivity(LD) and mode of anisotropy(MO)

Corresponding Author: Jasmohan S Bajaj, MD, MS, Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University and McGuire VA Medical Center, 1201 Broad Rock Boulevard, Richmond, VA, 23221, USA, Phone: (804) 675-5802, Fax: (804) 675 5816, jasmohan.bajaj@vcuhealth.org.

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continued to increase in the posterior internal capsule at both post-LT visits. On VBM, a continued increase was seen in basal ganglia grey matter between both post-LT visits was seen.

Conclusions—HRQOL and cognition continue to improve compared to pre-LT values up to 1 year post-LT, although the rate of improvement slows down after 6 months. Grey matter increase is steady over time at 1 year although changes in ammonia-related metabolites and white matter integrity improve at a slower pace at 1 year post-LT.

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric condition occurring in patients with liver failure. It manifests as deficits in motor function, coordination, psychomotor function, sleep-waking cycle and impaired thought process and cognition. Although the pathological mechanism of HE remains unclear, there is growing evidence that hyperammonemia and neuroinflammation may act synergistically to cause brain edema and neurotransmission disturbances leading to the neurological alterations in HE¹.

Magnetic resonance imaging (MRI) and spectroscopy (MRS) are noninvasive techniques that have been used for investigating brain biomarkers for HE². MRS provides information about the neurometabolic status³. High resolution T1-weighted images and diffusion tensor imaging (DTI) along with advanced analysis techniques such as Voxel-based morphometry (VBM) and Tract-based Spatial Statistics (TBSS) have been used to study the localized and global grey and white matter damage in cirrhosis respectively^{4–6}. DTI is based on quantitative assessment of the mobility of water molecules. It has been used to study the microstructural integrity of axon and brain edema in cirrhotic patients^{7–11}.

HE is presumed to be largely reversed after successful liver transplant (LT)³. However, there are variable results regarding cognitive changes long-term post-LT¹². While some studies have determined a continued improvement in cognition and daily function, others have shown a staggered course in those with pre-LT HE^{13,14}. Fewer studies have evaluated the role of multi-modal brain MRI changes over a longer time-period and longitudinal analyses of these MRI changes have also provided conflicting results^{15,16,33}. Cordoba et al reported a progressive improvement in Magnetization-Transfer ratio (marker for brain edema) and MR Spectroscopy between pre-LT, 1 month post-LT and 1 year post-LT without continued improvement in cognition at 1 year post-LT. Garcia-Martinez et al performed neuro-psychological assessments before and 6–12 months after LT. They also performed MRS and total brain volume assessment only after-LT to show a relationship of prior HE with post LT outcome. Lin et al showed improved extracellular cerebral edema and cognitive tests 6 to 12 months after transplant. They also showed a possible advancement of white matter demyelination in the temporal lobe. Our prior study showed that at 6 months post-LT there is a significant improvement in cognitive performance and health-related quality of life (HRQOL)¹⁷. This was accompanied by a significant improvement in white matter integrity and astrocytic consequences of hyperammonemia on multi-modal MRI¹⁷. The follow-up of that study, however, was limited to 6 months and did not include voxel-based morphometry. In this study, we evaluated cognitive, HRQOL tests and brain metrics, including brain volumes using VBM 1-year post-LT.

We hypothesized that there would be continued improvement in MRI metrics, cognition and HRQOL when comparing 1-year post-LT with 6-month post-LT, while remaining better compared to the pre-LT baseline.

MATERIALS AND METHODS

We prospectively included patients with cirrhosis listed for deceased solitary liver transplant at the VCU Medical Center from June 2011 to June 2015. Patients who were able to give informed consent who were between 21 to 65 years of age, able to tolerate multi-modal brain MRI and were followed over 1 year post-LT were included. We excluded those with severe depression (Beck Depression Inventory >20)¹⁸, who were not able to give informed consent, inability to tolerate or contraindications to brain MRI, dropout over 1 year post-LT, prior transplant, HIV infection or those who are listed under Priority 1 status.

At the pretransplant visit, the subjects were given the mini-mental status examination and were only allowed to proceed if it was ≥ 25 . After this we performed HRQOL assessment using the Sickness Impact Profile (SIP)¹⁹ which has a total of 12 domains, 2 dimensions and total score. Scores from Psychosocial and Physical dimensions and the total scores were calculated. A high total SIP score suggests poor HRQOL and vice versa. Cognitive testing was administered by a highly experienced and trained nurse (MBW) using 5 paper-pencil tests: The Psychometric Hepatic Encephalopathy Score (PHES) consists of 5 separate tests; the number connection test-A (NCT-A) and number connection test-B (NCT-B), the digit symbol test (DST), the serial dotting test (SDT), and the line drawing test (LDT). These tests evaluate attention, visuo-spatial perception, visuo-spatial construction, psychomotor speed and motor accuracy; i.e. domains that have been shown to be affected by HE. Compared to a healthy control population, the number of standard deviations impaired are calculated for each test and assigned points. An aggregate of < -4 points is considered impaired according to the local age- and education-adjusted norms. PHES is widely considered as the current “gold standard” in diagnosing cognitive impairments in cirrhosis at an early stage²⁰. Additionally, a block design test (BDT) was administered as a measure of visuo-spatial motor functioning²¹.

All subjects then underwent multi-modal brain MRI (details below). Subjects were then followed until liver transplantation and up to 12 months beyond that. The transplant surgery details, immuno-suppression regimen, hospitalizations, rejection episodes, and continued need for organ support such as dialysis was recorded. The first posttransplant visit was performed at 6 (± 3) months for patients who were alive and willing, and had not undergone a second transplant or were listed for another transplant (including renal transplant); and the second one was performed at 12 (± 4) months. At both post-LT visits, all procedures performed at the baseline visit, including brain MRI, were repeated.

MRI Acquisition

All images during the pre-LT, post-LT1 and post-LT2 sessions were acquired on a 1.5T Siemens Avanto (Erlangen, Germany) scanner using a quadrature birdcage RF head coil (Supplementary data).

MR Spectroscopy analysis—The choline (Cho), creatine (Cr), myo-inositol (mI) and glutamate+glutamine (Glx) complex peak areas were computed using a quantitative assessment of the metabolite concentration by means of LCModel software^{22,23}. These metabolites were chosen because of prior research showing cirrhosis related changes in these metabolites². Concentration ratios were computed with respect to creatine concentration. LCModel utilizes a basis set of reference *in vitro* MR spectra for all major metabolites to deduce absolute concentrations of corresponding compounds from *in vivo* MR brain spectra. The model corrects for residual eddy current and RF coil loading effects and allows for an estimate and subtraction of the spectral baseline nonlinearity which is normally present at the short TE used in this study^{22,23}.

Voxel-based morphometry (VBM)—Structural data was analyzed with FSL-VBM²⁴ an optimized VBM protocol²⁵ carried out with FSL tools²⁶. First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI 152 standard space using nonlinear registration. The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images were nonlinearly registered to this study-specific template and “modulated” to correct for local expansion (or contraction) due to the nonlinear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 2 mm. Finally, voxel wise GLM was applied using permutation-based nonparametric testing²⁷, correcting for multiple comparisons across space.

DTI analysis—Diffusion weighted images were processed using tools in FSL (FMRIB Software Library version 5, www.fmrib.ox.ac.uk/fsl^{28–30}). After visual inspection for signal dropout, artifacts and head motion, diffusion images were corrected for eddy current related distortions and simple head motion using affine registration to a reference volume. Diffusion tensor model was fitted voxel-wise to generate maps of fractional anisotropy (FA), mean diffusivity (MD), and longitudinal diffusivity (LD), radial diffusivity (RD) and Mode of Anisotropy (MO). Voxelwise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics³¹), part of FSL²⁶ (Supplementary data).

Statistical analysis—All statistical analyses were performed using the SPSS (version 22, SPSS Inc., Chicago, IL, USA) with appropriate tests. To assess the impact of time on the measures, a mixed linear model (MLM) was utilized to fit a repeated measures ANOVA model (RMANOVA). The mixed linear model is superior to a MANOVA model in that all observations available are utilized for analysis and this all subjects with data are utilized, not just completers. The MLM fit had one within subject factor (Time: pre-LT, post-LT1 and post-LT2) and an unstructured variance-covariance matrix was used to model the within subject variance. If there was a significant time effect, the posthoc comparisons of the time points were performed utilizing a Bonferroni correct to adjust for multiple comparisons. We also compared the completers (all 3 visits vs. noncompleters who had only 2 visits completed) with respect to HRQOL, cognitive testing and MRS parameters. Voxel-wise analyses such as TBSS and VBM require the number of subjects to be same at all timepoints to avoid introducing registration bias; therefore MLM could not be performed for these

parameters. HRQOL, cognitive testing, MRS, diffusion indices (FA, MD, LD, RD, MO) and grey matter density (VBM) were also compared before and after transplant by the two-tailed paired t-test between pre-LT, post-LT1 and post-LT2 sessions i.e. pre-LT vs. post-LT1, post-LT1 vs. post-LT2 and pre-LT vs. post-LT2 in the groups who had completed all 3 visits. Multiple comparison correction was done using Holms-Bonferroni method for all testing (except for voxel-wise analyses) at a significance level $\alpha=0.05$. Voxel-wise comparisons in TBSS and VBM were performed using nonparametric permutation testing using 5000 permutations test and the resultant maps were corrected for family-wise error (FWE < 0.05) followed by threshold-free cluster enhancement method.

The study protocol was approved by the VCU and Richmond VA Institutional Review Boards.

RESULTS

Patients

Forty-five patients with a median listing age of 54 years with median MELD score of 21 were included, who had been followed from pre-LT to post-LT status. Of these, eleven only had 1 post-LT MRI at 6 months, 5 were lost to follow-up and 6 had technical issues in brain MRI evaluation at 1 year. All patients were included in the MLM analysis for cognitive function, HRQOL and MR spectroscopy (table s1). In addition we studied the twenty-three patients with cirrhosis who had completed all 3 MRI sessions; pre-LT, post-LT1(6±2 months) and post-LT2(12±3 months). The median age was 56 years, median MELD was 26 and most were men (n=16). Etiology of cirrhosis was Hepatitis C virus (HCV) in 11 patients, Alcohol + HCV in 5 patients, Nonalcoholic fatty liver disease in 7 patients. Eight patients were listed for hepatocellular cancer, 11 patients had prior overt HE (all on lactulose and rifaximin) and 9 patients had impaired PHES suggestive of covert HE pre-LT (Table 1). When comparing the completer vs. noncompleter sample, there were no significant differences in median PHES score pre-LT (completer -4, noncompleter -4, p=0.4) or post-LT1 (completer -1, noncompleter, 0, p=0.5). There were no significant differences in pre-LT values of total SIP (completer 21.60 ± 14.8 vs noncompleter 22.6±13.9, p=0.82), psychological SIP domain (completer 19.49 ± 18.5 vs noncompleter 19.0±16.4, p=0.35) and physical SIP (completer 16.03 ± 14.3 vs noncompleter 15.7 ± 16.1, p=0.32). This trend was seen in the post-LT1 visit as well for total SIP (completer 15.0 ± 16.5 vs noncompleter 16.3 ± 4.5, p=0.42), psychological SIP (completer 13.54 ± 18.9 vs noncompleter 12.72±16.7, p=0.86) and physical SIP (completer 12.12 ± 15.9 vs noncompleter 11.83±17.6, p=0.26).

Peri and posttransplant course

Patients underwent LT a median of 6 months (2–10 months IQR) after listing. There were no HE-related admissions during the interim period but 6 were hospitalized due to ascites (n=3), and infections (n=3) after which they were re-activated on the list. The perioperative period for the twenty-three included subjects was uneventful, without early rejection, continuing organ failure or primary graft nonfunction, with a median hospital stay of 9 (IQR 4–21 days). The standard VCU immunosuppression regimen includes tapering steroids over a month with mycophenolate mofetil initiated peri-operatively. Calcineurin inhibition in the

form of tacrolimus is introduced at day 3 to achieve levels between 8–12 ng/ml in the first 3 months and between 5–10 ng/ml after that period depending on the cirrhosis cause.

Of the 23 patients, 7 required interventions prior to post-LT1 (2 with rejection requiring steroids in all and ATG in one, 2 with biliary complications necessitating interventions and 2 with delirium requiring a switch from tacrolimus to cyclosporine). Between post-LT1 and post-LT2 visits, 2 patients required biliary interventions with cholangitis and 1 developed *C. difficile* colitis. All these episodes were satisfactorily treated at least 2 months prior to the cognitive testing and MRI scans at both post-LT visits.

Regular tacrolimus trough levels were monitored throughout and all patients were within the proscribed trough range (10.1 ± 3.7 ng/ml average tacrolimus level prior to post-LT1 and 7.3 ± 3.2 ng/ml before post-LT2 visits).

None of the HCV patients had SVR pretransplant and did not receive any anti-HCV therapy before the posttransplant testing. No antipsychotic medications were initiated and there was no evidence of impaired comprehension (MMSE was >25), tremors or focal neurological deficits at both post-LT visits. There was a significant improvement in laboratory, cognitive and HRQOL between pre and both post-LT visits using paired t-tests for the completers and the MLM (Table 2, table s1)

HRQOL and Cognitive testing

Total SIP and its components significantly decreased from pre-LT to post-LT1 session without further changes during the post-LT2 session (Table 2). Similarly, there was significant increase in PHES scores from pre-LT to post-LT1. Both SIP and PHES scores did not change significantly between post-LT1 and post-LT2 (Figure 1A/B). When all patients were considered, this trend remained similar with total SIP and the 2 domains (table s1).

MR Spectroscopy

There was a significant increase in Cho/Cr and ml/Cr and decrease in Glx/Cr in the post-LT1 session with no further improvement in the post-LT2 session in RPWM, PGM and ACC (Figure 2 A–C) using the completer sample. Post-LT2 measures were significantly better than pre-LT. Using the MLM, this trend was again seen.

Diffusion Tensor Imaging

We found no significant differences in diffusion metrics between post-LT1 and post-LT2 sessions. Compared to pre-LT session both post-LT1 and post-LT2 sessions showed a significant increase in LD and MO in the posterior limb of the internal capsule (Figure 3) with concurrent increase in MD without any significant changes in FA or RD. Increase in LD was also observed in the Splenium of the corpus callosum in post-LT1 and post-LT2 compared to pre-LT. (Table 3).

Voxel-based morphometry

Six months after transplant (post-LT1), we found significant increase in grey matter volume in brain regions within the basal ganglia, anterior cingulate cortex, frontal and temporal gyri

and decrease in grey matter volume in the thalamic regions. The grey matter volume continued to increase in the basal ganglia alone twelve months (post-LT2) after transplant (Figure 4A–B; Tables 4–6).

Discussion

Several studies have demonstrated cognitive recovery after LT^{15–17,32}. However, the timeline of these improvements has been variable. In this study, we investigated the integrated trajectory of improvement in HRQOL, cognition and brain metabolic and structural components over the course of a year post-LT, which is novel for this population. While the analyses between pre-LT and 6 months have been published, we believe that this paper adds significantly to our knowledge. Specifically the novel information is related to (a) Length of follow-up over 1 year and (b) Use of voxel-based morphometry and (c) use of voxel-wise analysis of Diffusion tensor imaging instead of regions-of-interest based analysis used in the prior study. The 1-year time-point is important because it gives clinicians a clearer picture as to what to expect cognitively and functionality-wise in patients. These patients at 1 year are more prone to post-LT metabolic sequelae and potential recurrence of diseases that caused the cirrhosis. Therefore extending findings over time and noting improvement compared to the pre-LT baseline is an important and novel translational question. Voxel-based morphometry is critical in determining volumetric changes, which indeed continued to change at all time points. The voxel-based analysis helps in localization and determining the shape of the diffusion tensor in greater detail compared to the ROI-based analysis.

The potential mechanism by which hyperammonemia and neuro-inflammation may cause neurodegenerative effects is as follows: Ammonia crosses the blood-brain barrier and is metabolized by astrocytes into glutamine, which is a precursor of glutamate and gamma-hydroxybutyric acid (GABA): the main excitatory and inhibitory neurotransmitters respectively³³. Increased astrocytic glutamine causes an influx of water leading to astrocytic swelling and brain edema. In chronic liver disease, the astrocyte compensates for excess glutamine by releasing other metabolites to the extracellular environment. The presence of ammonia, lactate and glutamate can activate the microglia, the macrophages of the brain, which then release a wide range of proinflammatory cytokines and chemokines¹. Sustained release of cytokines and chemokines may compromise the blood-brain barrier which in turn may further expose the interstitial space to neurotoxins such as ammonia and peripheral immune cells ultimately leading to neurodegeneration³. Therefore, multi-modal MRI techniques are well suited for studying these brain changes due to liver disease and evaluating the efficacy of treatments such as liver transplantation.

Posttransplant we saw an expected decrease in Glutamate+Glutamine and increase in myoInositol and choline in all 3 ROIs studied indicating a reversal of hyperammonemia related changes in glutamine production and compensatory behavior of the other metabolites. These results suggest a decrease in low grade brain edema after transplant^{16,17,34} that continues to evolve favorably between 6 months and 1 year post-LT. After LT, DTI analysis showed an increase in linear diffusivity and mode of anisotropy in the posterior limb of the internal capsule. This indicates a shift from diffusivity of water molecules from a planar direction to an increasingly linear direction, suggesting an increase

in axonal integrity. A significant portion of the posterior limb of internal capsule contains the fibers of the corticospinal tract which contain axons originating from frontal and parietal areas and are involved in voluntary movement and sensory functions³⁵. This recovery continued at 1 year after LT, which even though was not statistically significant, implies that this process evolves over time differently in different brain white-matter regions. This was accompanied by an LD increase in the splenium region of the corpus callosum may have been associated with improved interhemispheric information transfer in study subjects.

The novel VBM analysis revealed a reversal of grey matter volume changes post-LT that continued up to 1 year. Previous studies^{5,32} reported a significant decrease in grey matter density in cirrhosis was indicative of increasing atrophy and worse hepatic function, likely due to hyperammonemia¹⁵. Six months after transplant, our results indicate an increase in grey matter volume in basal ganglia, a group of subcortical nuclei responsible primarily for motor control and learning and due to its rich connections to both cortical and subcortical structures, ultimately impacts cognitive function, behavior, and emotions^{36,37}. Other brain regions associated with increased grey matter volume at 6 months posttransplant include the anterior cingulate cortex, involved in attention³⁸, and emotional suffering^{39,40}; temporal gyri, associated with language and memory and frontal gyri involved in working memory and inhibitory control³⁵. Thus, the multi-modal brain imaging findings complement the cognitive improvement on tests that assess these domains. We also found a decrease in thalamic and hippocampal volumes after transplant. The increase in thalamus grey matter has been previously reported in cirrhosis patients⁵. Although the precise reasons for thalamic size change remains uncertain, studies suggest that increased thalamic volume is a compensatory response to a corresponding decrease in basal ganglia grey matter regions. A possible mechanism for this dynamic reflects a redistribution of cerebral blood flow from the cortices to the thalamus in cirrhotic patients. These blood flow changes facilitate increased glucose and ammonia metabolism in the thalamus, with a corresponding metabolism reduction in other cortical regions. The increased blood flow and metabolism in the thalamus might be associated with neuronal and/or glial hypertrophy or hyperplasia, resulting in increased volume. Given the rich connections between hippocampal and thalamic structures the larger hippocampal volumes seen pre-LT may reflect the same dynamic. Importantly, we found continued improvement in grey matter density until 1 year post LT in basal ganglia regions. This continued increase in basal ganglia density was associated with improvement in cognition and HRQOL, which despite not reaching significance compared to the 6-month mark, showed a continuous trajectory of improvement. Specifically, improvements in SIP values continued and most individual cognitive tests improved at the 1 year time-point.

Overall our results suggest that post-LT cirrhosis patients evolve at different trajectories over time post-LT with most dramatic differences between pre-LT and 6 months and continued improvement thereafter with regard to their cognitive and HRQOL. This was found when all comers were analyzed and also using only the completer sample. This was complemented by specific structural and metabolic changes reflecting further recovery of function even after 6 months. In summary, patients at 1 year post-LT enjoyed better cognitive function, HRQOL, and, based on multi-modal MRI brain imaging measures, improved cortical structure and function.

One of the limitations of the study was that patients who had an eventful post-LT course did not follow-up for repeat MRI sessions. Therefore, the interpretation of these results should be limited to patients whose course was stable. Another limitation of this study was our relatively small sample size. The choice of waiting 6 months for the first follow-up post-LT may be considered a limitation. At the 1-month follow-up, Cordoba et al¹⁵ found that some metrics were either worse or at pre-LT levels and they attributed this to neurological effects of surgery and postsurgical management and slower evolution of certain brain metrics and cognitive function relative to others. Therefore, while designing the study, we wanted to exclude the potentially confounding neurological effects of surgery and early postoperative complications and management on our results, while giving sufficient time for all domains to potentially evolve. Finally, due to our sample-size we could not separately evaluate the post-LT trajectory for patients with prior HE episodes, patients with pre-LT covert HE and patients based on etiology. Although prior studies have shown neurological differences between these sub-groups, the brain regions involved are similar with only magnitude of differences being variable. More importantly, hyperammonemia and neuro-inflammation are still the factors driving the cognitive impairments which LT potentially addresses.

We conclude that the improvements seen in cognition, quality of life, brain metabolites and brain structures at 6 months post-LT improve, but at a slower pace, between 6 months and 12 months post-LT and remain significantly better compared to the pre-LT baseline. These results provide insight to guide clinicians while counseling patients and caregivers regarding the stability of functional improvement after liver transplant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HRQOL	health-related quality of life
HE	hepatic encephalopathy
PHES	Psychometric hepatic encephalopathy score
DTI	diffusion tensor imaging
FA	fractional anisotropy
MD	mean diffusivity
LD	linear diffusivity
RD	radial diffusivity
MO	mode of anisotropy

Cho	choline
Cr	creatine
mI	myo-inositol
Glx	glutamate+glutamine
ROI	regions of interest
MLM	mixed linear model
RMANOVA	repeated measures analysis of variance
VBM	voxel-based morphometry

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Figure 1. Improvement on Sickness Impact Profile (Figure 1A) and Psychometric hepatic encephalopathy score (PHES Figure 1B) seen at 6 months (post-LT1) after transplant evolves differently 12 months after transplant (post-LT2). SIP Sickness Impact profile, Total SIP = Psychosocial + Physical components, High SIP indicates poor quality of life, PHES Psychometric Hepatic Encephalopathy Score, Low PHES indicates poor performance.

Figure 2.

Brain metabolite concentration ratios changes between pre-LT, post-LT1 and post-LT2 sessions. ^{**} $p < 0.05$, ^{**} $p < 0.005$ pre-LT vs post-LT1, [†] $p < 0.05$, ^{††} $p < 0.005$ pre-LT vs post-LT2. Glx/Cr decreases and ml/Cr, Cho/Cr increase post-LT1 without further improvement at post-LT2; Cho: Choline; Glx: Glutamate+Glutamine; ml: myoInositol; Cr: Creatine; RPWM: Right parietal white matter; PGM: Posterior gray matter; ACC: Anterior cingulate gyrus.

Figure 3.

Significant results from Tract-Based Spatial Statistics (TBSS) analysis for (A) Mode of Anisotropy(MO) and (B) Linear Diffusivity (LD) overlaid on mean FA skeleton map (Green). Family-wise error corrected p-value < 0.05 was used as the threshold for MO, LD maps.

(A) pre-LT > post LT1 (Red-Yellow) and post-LT1 > pre-LT (Blue-LightBlue); t-score = 2.7 – 6

(B) post-LT1 > post-LT2 (Red-Yellow); t-score = 2.7 – 6

Figure 4.

Voxel Based morphometry (VBM) between pre-LT, post-LT1 and post-LT2 sessions. (A) pre-LT vs. post-LT1: Significant increase is seen in grey matter volume in the basal ganglia, anterior cingulate cortex, frontal and temporal gyri accompanied by significant decrease in the thalamic regions. (B) post-LT1 vs. post-LT2: Grey matter volume continues to increase in the basal ganglia in the post-LT2 session. R right, L left, A anterior, P posterior, MNI Montreal Neurological Institute x/y/z standard space coordinates in mm, Family-wise error rate $p < 0.05$

Table 1

Demographic information of the sample

	(n=23)
Age (mean±SD)	56.8 ± 7.6
Years of Education (mean±SD)	12.8±1.6
Gender (men/women)	16/7
Race (Caucasian/African-American/Hispanic)	14/5/4
MELD score (mean±SD)	22.6±8.7
Number with prior Overt HE (number)	11
Number with impaired PHES (number)	9

Table 2

Comparison of laboratory, functional and cognitive results across visits

	Pre-LT	Post-LT1	Post-LT2
Hemoglobin (g/dL)	9.7±3.4	11.8±2.3 *	12.3±3.4 †
WBC Count (/mm ³)	5.8±2.8	3.7±2.8 *	3.9±3.7 †
INR	1.7±0.9	1.2±0.4 *	1.1±0.6 †
Total Bilirubin (mg/dL)	6.3±11.3	1.0±1.2 *	0.7±0.9 †
Serum albumin (g/dL)	2.9±0.7	3.8±0.8 *	3.8±1.2 †
Serum creatinine (mg/dL)	1.9±2.1	1.5±1.3	1.3±1.6
Serum sodium (meq/L)	133.5±10.3	141.0±3.1 *	142.6±3.8 †
Number connection-A (seconds)	41.21 ± 17.2	32.67 ± 15.0 *	30.52 ± 9.3 †
Number connection-B (seconds)	120.07 ± 66.7	82.96 ± 32.9 *	75.52 ± 26.5 †
Block design (raw score) *	28.14 ± 13.0	33.89 ± 13.9 *	35.48 ± 11.7 †
Digit symbol (raw score) *	50.97 ± 13.2	58.89 ± 14.0**	62.76 ± 14.6 †
Serial dotting test (seconds)	71.28 ± 24.1	58.07 ± 13.4**	59.22 ± 19.3 †
Line tracing time (seconds)	105.34 ± 43.7	87.97 ± 32.2**	95.43 ± 27.0
Line tracing errors	33.97 ± 33.9	32.63 ± 28.4	23.86 ± 19.6 †
PHES total score median (IQR)	-4 (-2, -10)	-1 (0, -4) *	-0.6 (1, -3)
Sickness Impact Profile Total	21.60 ± 14.8	15.00 ± 16.5 *	11.56 ± 13.2 †
Sickness Impact Profile Psychosocial	19.49 ± 18.5	13.54 ± 18.9 *	10.90 ± 16.7 †
Sickness Impact Profile Physical	16.03 ± 14.3	12.12 ± 15.9*	6.84 ± 10.9 †

* p< 0.05 pre-LT vs. post-LT1,

† p<0.05 pre-LT vs. post-LT2, No changes were seen between post-LT1 and post-LT2. A significant improvement in most cognitive tests and laboratory values was seen in all patients after transplant. A high score on block design and digit symbol test indicates good performance; the reverse is true for other cognitive tests. Similar changes were found on repeated measures analysis.

Table 3

TBSS analysis revealed white matter regions with significant changes after LT.

	R/L	t-score	MNI (mm)		
MO: postLT1>preLT					
Cluster 1: 183 voxels			x	y	z
Posterior limb of internal capsule	R	5.91	20	-13	-1
Posterior limb of internal capsule	R	4.16	19	-9	2
MO: postLT2>preLT					
	R/L	t-score	MNI (mm)		
Cluster 1=445 voxels			x	y	z
Cerebral Peduncle	L	6.44	-14	-10	-8
Posterior limb of internal capsule	L	5.52	-23	-20	4
Retrolenticular part of internal capsule	L	4.73	-24	-21	6
Cluster 2=327 voxels					
Posterior limb of internal capsule	R	6.01	19	-12	-1
Posterior limb of internal capsule	R	4.44	22	-11	10
Cerebral Peduncle	R	4.4	16	-13	-11
LD: postLT1>preLT					
	R/L	t-score	MNI (mm)		
Cluster 1: 6072 voxels			x	y	z
Inferior fronto-occipital fasciculus	R	7.02	25	-19	48
Posterior limb of internal capsule	R	6.24	23	-17	2
Anterior Limb of internal capsule	R	5.93	18	-1	11
Cluster 2: 6060 voxels					
Posterior limb of internal capsule	L	7.59	-23	-14	11
Cerebral Peduncle	L	6.96	-17	-13	-6
Corticospinal tract	L	6.88	-28	-21	34
Posterior limb of internal capsule	L	6.78	-19	-6	9
LD: postLT2>preLT					
	R/L	t-score	MNI (mm)		
			x	y	z

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	R/L	t-score	MNI (mm)		
MO: postLT1>preLT					
Cluster 1: 13725 voxels					
Posterior limb of internal capsule	L	8.47	-23	-16	9
Posterior limb of internal capsule	R	7.55	19	-12	-4

TBSS: tract-based spatial statistics, LT: liver transplant, LD: linear diffusivity, MO: Mode of Anisotropy

Table 4

Regions with significant grey matter volume increase in post-LT1 compared to pre-LT

post-LT1 > pre-LT	R/L	t-score	MNI (mm)		
			x	y	z
<i>Cluster 1: 3899 voxels</i>					
Accumbens	L	8.39	-6	4	-4
Accumbens	R	7.93	8	10	-6
Accumbens	L	7.33	-6	8	-4
Putamen	L	7.31	-18	10	-4
Putamen	R	6.57	24	16	-6
Amygdala	L	5.76	-28	-10	-12
Caudate	L	3.8	-12	18	8
Caudate	R	4.44	16	14	8
<i>Cluster 2: 568 voxels</i>					
Cingulate gyrus, anterior division	R	4.98	6	34	12
Cingulate gyrus, anterior division	R	4.69	4	38	22
Superior Frontal gyrus	L	4.43	-2	38	44
Paracingulate gyrus	L	4.36	-6	44	20
Cingulate gyrus, anterior division		4.13	8	42	8
Frontal Pole	L	4.07	-12	50	32
<i>Cluster 3: 122 voxels</i>					
Middle Temporal Gyrus (anterior division)	L	5.21	-58	0	-18
Inferior Temporal Gyrus (anterior division)	L	5.07	-52	-10	-28
Superior Temporal Gyrus (anterior division)	L	4.01	-46	0	-22
<i>Cluster 4: 117 voxels</i>					
Amygdala	R	7.96	26	-6	-10
<i>Cluster 5: 97 voxels</i>					
Brain Stem	R	4.46	2	-28	-10

post-LT1 > pre-LT	R/L	t-score	MNI (mm)		
			x	y	z
<i>Cluster 1: 3899 voxels</i>					
Brain Stem	L	4.35	-2	-38	-8
<i>Cluster 6: 50 voxels</i>					
Occipital Pole	R	5.41	32	-98	-6
Occipital Pole	R	3.67	32	-92	-2
<i>Cluster 7: 18 voxels</i>					
Frontal Pole	L	4.99	-40	38	-2
Inferior frontal gyrus, pars triangularis	L	4.67	-38	34	0
Inferior frontal gyrus, pars triangularis	L	4.1	-44	30	6
<i>Cluster 8: 10 voxels</i>					
Lateral Occipital Cortex, inferior division	R	4.02	30	-88	-8
<i>Cluster 9: 9 voxels</i>					
Intracalcarine cortex	L	5.69	-16	-78	4
<i>Cluster 10: 8 voxels</i>					
Occipital Fusiform gyrus	R	3.43	20	-90	-4
<i>Cluster 11: 3 voxels</i>					
Lingual gyrus	L	4.92	-14	-88	-8

Table 5

Regions with significant grey matter volume decrease in post-LT1 compared to pre-LT

pre-LT > post-LT1	R/L	t-score	MNI (mm)		
			x	y	z
<i>Cluster 1: 1805 voxels</i>					
Thalamus	R	8.72	10	-28	10
Parahippocampal gyrus, posterior division	R	7.89	16	-36	-6
Thalamus	R	7.17	4	-4	8
Thalamus	L	6.75	-10	-20	4
Thalamus	L	6.4	-16	-28	12
Hippocampus	L	5.39	-20	-38	0
Hippocampus	R	5.22	26	-36	-2

LT: Liver transplant, R/L: right/left, MNI: Montreal Neurological Institute, Family-wise error rate $p < 0.05$

Table 6
Regions with significant grey matter volume increase in post-LT2 compared to post-LT1

post-LT2 > post-LT1	R/L	t-score	MNI (mm)		
			x	y	z
<i>Cluster 1: 365 voxels</i>					
Putamen	L	6.5	-22	10	-2
Putamen	L	6.37	-30	4	2
Putamen	L	5.74	-24	0	4
Putamen	L	4.62	-30	-6	4
Putamen	L	4.3	-30	-10	2
<i>Cluster 2: 9 voxels</i>					
			x	y	z
Caudate	R	5.05	16	22	-4

R right, L left, MNI Montreal Neurological Institute standard space coordinates, Family-wise error rate $p < 0.05$