Combining arterial-spin labeling with functional magnetic resonance imaging measurement for characterizing patients with minimal hepatic encephalopathy

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Aim: Our objective is to explore key changes in brain functions in relation to minimal hepatic encephalopathy (MHE). We incorporated both resting-state functional magnetic resonance imaging (fMRI) and arterial spin labeling (ASL) to enhance the detection of MHE.

Methods: We undertook fMRI scanning for 56 MHE patients and 66 healthy controls. Region functional connectivity was carried out to assess the connectivity status between pairs of regions among 90 brain regions. Additionally, blood flow (BF) status was measured by ASL for all subjects. Spearman’s correlation test was implemented to identify any correlation among z-values, results from number connection test type A, and digit symbol tests. Finally, the receiver operating characteristic curve was generated for assessing the accuracy of BF in MHE diagnosis.

Results: The corresponding functional connectivity was significantly different between MHE and control groups in 15 regions. For MHE patients, BF showed an increasing pattern in regions of interest. Blood flood in the putamen was positively correlated with number connection test type A neuropsychological performance, whereas it was negatively correlated with the digit symbol test. Blood flood in the right putamen showed the highest value of area under the curve with a sensitivity of 85.7% and specificity of 89.4%.

Conclusion: Connectivity impairment resulting from ganglia-thalamo-cortical circuits may play important roles in mediating the development of MHE patients. An increase in the BF, particularly in the right putamen, may be considered as evidence for the presence of MHE.

Key words: arterial spin labeling, blood flow, functional connectivity, minimal hepatic encephalopathy, resting-state functional MRI
Arterial-spin labeling and functional MRI in MHE

METHODS

Subjects

Patients with hepatic cirrhosis were selected if they were diagnosed with clinical, histological, and biochemical symptoms of liver cirrhosis at the Second Hospital of Hebei Medical University (Shijiazhuang City, China) between January 2012 and August 2014. Patients who had other brain diseases, including severe HE, trauma, cerebral infarct, or tumor affecting brain functions, were excluded. Patients with MHE were classified according to psychometric hepatic encephalopathy score (PHES) performance identified as the gold standard, which mainly comprised two psychometric tests, connection test type A (NCT-A) and the digit symbol test (DST). Patients who had a psychometric test score less than 4 points were considered as MHE patients. Finally, 56 MHE patients were included in this study, including 48 men and 8 women with an average age of $53.9 \pm 9.8$ years. Sixty-six healthy subjects without any neurological or psychiatric disease history were also recruited from the same hospital, who took a health examination during the same time, including 40 men and 26 women with an average age of $51.3 \pm 9.7$ years. This study was approved by the ethics committee of the Second Hospital of Hebei Medical University. All subjects provided signed informed consent before neuropsychological test or MRI examinations and all experiments were carried out according to relevant approved guidelines and regulations of the Second Hospital of Hebei Medical University.

Laboratory examinations

Venous blood (4 mL) was collected following an overnight fast to measure the level of blood ammonia in MHE patients. The blood biochemistry tests were carried out in the 24 h before MRI examination in all patients. To evaluate the severity of cirrhosis, Child–Pugh score was analyzed including five clinical parameters: encephalopathy, ascites, prothrombin time, and levels of serum albumin and bilirubin. The score for each variable ranged from 1 to 3 and patients were classified as A (5–6 score), B (7–9 score), or C (10–15 score).

Magnetic resonance imaging

All participants were scanned with a 3T MRI scanner (Vantage Titan; Toshiba, Tokyo, Japan). A head receive/transmit coil was used to reduce head motion. Resting-state functional MR images were acquired using a gradient-recalled imaging program (repetition time = 2000 ms/30 ms; 250 volumes; flip angle = 90°; view field = 240 × 240 mm; section thickness = 4 mm;
matrix = 64 × 64; slices = 30). The perfusion images of ASL were obtained using the pulsed ASL technique (Philips, Amsterdam, Netherlands) following the parameters: matrix = 64 × 64 × 11, view field = 256 × 256 mm, bandwidth = 2232 Hz/pixel, repetition time = 2500 ms, flip angle = 90°, echo time = 11 ms, interslice space = 2 mm, slice thickness = 8 mm, TI1 = 1800 ms, TI2 = 700 ms. The ASL data were analyzed using homemade software written with interactive data language. This processing program normalized the homophase data of pixels to the Günther model for language. This processing program normalized the homemade software written with interactive data language. This processing program normalized the homophase data of pixels to the Günther model. The ASL data were analyzed using the lock-looker ASL acquisition, and blood flow (BF) was also calculated with equation [A2] from Günther et al. This program automatically calculated the M0 image with ASL data and CBF maps were formed after image analysis.

Preprocessing of fMRI data

The preprocessing of fMRI data was operated with DPARSF software (http://rfmri.org/DPARSF). The first 10 volumes were deserted, and the other 240 measures were analyzed to evaluate translation differences and head motion between MHE patients and controls. Image data from three MHE patients and two healthy subjects were discarded for either excessive translation or head motion. Finally, 53 MHE patients and 64 healthy subjects were included in the analysis of fMRI data. There were no differences in rotation, translation, or motion spike numbers between MHE patients and healthy controls (P > 0.05). Functional data were normalized to a standard template (3 × 3 × 3 mm³ from the Montreal Neurological Institute space). Linear trend was abandoned and band pass filtering (0.01–0.08 Hz) was used to decrease the impacts of low frequency drift and high frequency physiological noise with REST1.8 software (http://www.restfmri.net/forum/REST_V1.8). Meanwhile, several variables including signals from global mean, cerebrospinal fluid, and white matter, as well as head motion parameters, were removed from the fMRI data using a linear regression method.

Functional connectivity analysis

For every subject, FC was generated by calculating the correlation coefficient (r score) among each pair of DMN regions. Correlation coefficients were converted to z values with Fisher’s r-to-z transform in order to assess the population correlation coefficient.

Statistical analysis

Measurement data were expressed as median or mean ± standard deviation and the corresponding statistical analysis includes the t-test, χ²-test, or rank-sum test. For each group, the t-test was carried out to compare individual z-values among the corresponding 15 pairs of DMN connectivity. In order to examine differences in FC between the MHE and control groups, the t-test was carried out again. Spearman’s correlation was used to analyze the correlation between z-values and BF with neuropsychological performance (NCT-A and DST). Finally, receiver operating characteristic (ROC) curves were plotted to determine the accuracy of BF with respect to MHE diagnosis. All statistical analyses were undertaken using SPSS 21.0 software (IBM, New York, NY, USA) and P < 0.05 was considered for statistically significant difference.

RESULTS

Clinical data and neuropsychological tests

No differences were found in age, gender, or education between MHE patients and control subjects (P > 0.05). However, compared to control subjects, MHE patients had poorer neuropsychological performance (P < 0.05), which was reflected by longer time of NCT-A test and a lower DST score (Table 1). Control subjects had a DST score of 47.1 ± 9.9 and NCT-A score of 44.7 ± 11.9. Both DST and NCT-A scores were applied to classify MHE patients.

Table 1 Demographical characteristic and clinical data of study subjects

| Variable                   | Control     | MHE patients | P-value |
|----------------------------|-------------|--------------|---------|
| Age, years                 | 51.3 ± 9.7  | 53.9 ± 9.8   | 0.145   |
| Sex                        |             |              | 0.253   |
| Male                       | 40          | 48           |         |
| Female                     | 26          | 8            |         |
| Education, years           |             |              | 0.997   |
| <1                         | 4           | 3            |         |
| <6                         | 9           | 8            |         |
| <12                        | 36          | 30           |         |
| ≥12                        | 17          | 15           |         |
| Etiology                   | N/A         |              |         |
| Posthepatic cirrhosis      | 41          |              |         |
| Biliary cirrhosis          | 6           |              |         |
| Schistosomal cirrhosis     | 2           |              |         |
| Alcoholic cirrhosis        | 2           |              |         |
| Budd-Chiari syndrome       | 1           |              |         |
| Unknown etiology           | 4           |              |         |
| DST                        | 47.1 ± 9.9  | 25.9 ± 8.3   | <0.001  |
| NCT-A                      | 44.7 ± 11.9 | 72.9 ± 28.3  | <0.001  |
| Child–Pugh scale, A / B / C| 28/25/3     | N/A          |         |
| Ammonia level, mol/L       | 47.1 ± 22.1 | N/A          |         |

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Finally, 56 patients (48 men and 8 women; mean age, 53.9 ± 9.8 years) with an average NCT-A score of 72.9 ± 28.3 and DST score of 25.9 ± 8.3 were identified as MHE (Table 1). The Child–Pugh score in MHE patients was A (28), B (25), and C (3) and the blood ammonia level was 47.1 ± 22.1 μmol/L (Table 1). Posthepatic cirrhosis topped the etiology of MHE patients studied, accounting for three-quarters of the whole population, while biliary cirrhosis, schistosomal cirrhosis, alcohol cirrhosis, and Budd–Chiari syndrome were minor causes.

Functional connectivity

There were 15 brain regions in which the corresponding FC was significantly different between the MHE and control groups (P < 0.05; Table 2). Among the 15 connectivities, 12 (80%) were much weaker in MHE patients than in healthy controls, and 3 connectivities were significantly stronger in MHE patients compared with controls. Of the 15 edges, 9 edges with weaker connectivity (60%) were all correlated with subcortical regions (bilateral pallidums, bilateral putamens, and bilateral thalami); the remaining 6 connectivities (40%) were significantly different in cortical regions between the two groups.

Among the 15 connectivities, 7 edges were positively or negatively correlated with the DST scores (P < 0.05, Table 3). Also among the 7 connectivities, those positively correlated with DST were right globus pallidus–left thalamus (r = 0.674, P = 0.020), right lobus insularis–left thalamus (r = 0.337, P = 0.011), and left anterior cingulate cortex–left putamen (r = 0.648, P = 0.026). There were 4 connectivities that were negatively correlated with DST (right parahippocampal gyrus–left putamen (r = −0.317, P = 0.017), right precuneus–left globus pallidus (r = −0.473, P < 0.001), left pars triangularis–left postcentral gyrus (r = −0.325, P = 0.015), and left postcentral gyrus–right supra marginal gyrus (r = −0.283, P = 0.034).

Blood flow is increased in regions of interest of MHE patients

The perfusion images of CBF on MHE patients are shown in Figure 1. Blood flow was increased in our regions of interest (ROI) in patients with MHE, as shown in Figure 2. Blood flow in caudatum was 24.67 ± 2.85 mL/min per 100 g in controls and 28.94 ± 3.35 mL/min per 100 g in MHE patients, which was statistically significantly high compared to the control group (P < 0.001). Blood flow in globus pallidus were 37.74 ± 4.85 and 76.23 ± 8.20 mL/min per 100 g in healthy controls and in MHE patients with significant difference between the two groups (P < 0.001). In thalamus, BF was still higher in MHE patients (35.44 ± 3.84 mL/min per 100 g) than in controls (21.97 ± 1.50 mL/min per 100 g in controls). Similarly, BF in putamen was higher in MHE patients (32.15 ± 3.6 mL/min per 100 g) compared with control (20.40 ± 1.05 mL/min per 100 g). Meanwhile, BF in putamen was positively correlated with NCT-A neuropsychological performance (r = 0.391, P = 0.024; Table 4) but negatively correlated with DST (r = −0.358, P = 0.017; Table 4).

Table 2 Significant changes in functional connectivity among patients with minimal hepatic encephalopathy (MHE) and controls

| Functional connectivity | Z-value in controls | Z-value in MHE patients | P-value |
|-------------------------|---------------------|-------------------------|---------|
| R. lobus insularis–L. thalamus | 0.26 ± 0.22 | −0.05 ± 0.25 | <0.001 |
| L. anterior cingulate cortex–L. putamen | 0.35 ± 0.185 | 0.10 ± 0.17 | <0.001 |
| R. anterior cingulate cortex–L. caudatum | 0.54 ± 0.26 | 0.22 ± 0.23 | <0.001 |
| R. anterior cingulate cortex–L. putamen | 0.34 ± 0.18 | 0.09 ± 0.18 | <0.001 |
| R. parahippocampal gyrus–L. caudatum | −0.19 ± 0.17 | −0.02 ± 0.215 | <0.001 |
| R. parahippocampal gyrus–L. putamen | −0.37 ± 0.23 | −0.11 ± 0.195 | <0.001 |
| R. precuneus–L. globus pallidus | −0.39 ± 0.24 | −0.13 ± 0.19 | <0.001 |
| R. postcentral gyrus–R. thalamus | −0.31 ± 0.22 | −0.01 ± 0.23 | <0.001 |
| R. globus pallidus–L. thalamus | 0.54 ± 0.26 | 0.24 ± 0.16 | <0.001 |
| L. middle frontal gyrus–L. precuneus | −0.40 ± 0.22 | −0.14 ± 0.19 | <0.001 |
| L. middle frontal gyrus–R. precuneus | −0.42 ± 0.21 | −0.17 ± 0.19 | <0.001 |
| L. pars triangularis–L. postcentral gyrus | −0.29 ± 0.25 | 0.03 ± 0.24 | <0.001 |
| L. entorhinal cortex–R. middle frontal gyrus | 0.33 ± 0.22 | 0.43 ± 0.25 | <0.001 |
| R. entorhinal cortex–R. middle frontal gyrus | 0.47 ± 0.28 | 0.87 ± 0.30 | <0.001 |
| L. postcentral gyrus–R. supramarginal gyrus | −0.01 ± 0.21 | 0.38 ± 0.27 | <0.001 |

L, left; R, right.
To evaluate whether BF in ROI may be useful to diagnose MHE patients, the ROC curve was used to determine the area under the curve, cut-off value, and sensitivity and specificity for detecting MHE (Fig. 3). The BF in right putamen had the highest area under the curve of 0.875 with 85.7% sensitivity and 89.4% specificity, and the cut-off value was 25.66 mL/min per 100 g (Fig. 3b), which could be the potential biomarker for MHE detection. The BF values in right thalamus (Fig. 3a), left caudatum (Fig. 3c), and right globus pallidus (Fig. 3d) had both higher sensitivities and specificities (all >0.8) to distinguish MHE patients from controls.

**DISCUSSION**

Approximately 30–84% of patients with MHE also have cirrhosis; therefore, it was quite reasonable to believe that cirrhosis could partially contribute to the occurrence of MHE. As a matter of fact, until now there have been four confirmed pathogenesis of MHE, including functional disorders of the blood–brain barrier, reduced energy metabolism, production of neurotoxins within the gut, and altered emissions of cerebral neurotransmission. It was noteworthy that gut-derived neurotoxins might enable cirrhosis patients to develop MHE, in that abnormally elevated manganese concentrations were

| Functional connectivity                  | NCT-A correlation coefficient | NCT-A P-value | DST correlation coefficient | DST P-value |
|----------------------------------------|------------------------------|---------------|-----------------------------|-------------|
| R. lobarus insularis–L. thalamus       | 0.212                        | 0.117         | 0.337                       | 0.011       |
| L. anterior cingulate cortex–L. putamen| 0.017                        | 0.902         | 0.648                       | 0.026       |
| R. anterior cingulate cortex–L. caudatum| 0.014                       | 0.917         | -0.057                      | 0.675       |
| R. anterior cingulate cortex–L. putamen| 0.014                       | 0.919         | -0.055                      | 0.686       |
| R. parahippocampal gyrus–L. caudatum   | 0.016                       | 0.909         | -0.060                      | 0.662       |
| R. parahippocampal gyrus–L. putamen    | 0.082                       | 0.547         | -0.317                      | 0.017       |
| R. precuneus–L. globus pallidus        | 0.016                       | 0.905         | -0.473                      | <0.001      |
| R. postcentral gyrus–R. thalamus       | 0.014                       | 0.917         | -0.057                      | 0.675       |
| R. globus pallidus–L. thalamus         | -0.058                      | 0.882         | 0.674                       | 0.020       |
| L. middle frontal gyrus–L. precuneus   | 0.016                       | 0.905         | -0.056                      | 0.682       |
| L. middle frontal gyrus–R. precuneus   | 0.019                       | 0.887         | -0.055                      | 0.688       |
| L. pars triangularis–L. postcentral gyrus| 0.067                       | 0.625         | -0.325                      | 0.015       |
| L. entorhinal cortex–R. middle frontal gyrus| 0.014                       | 0.917         | -0.058                      | 0.673       |
| R. entorhinal cortex–R. middle frontal gyrus| 0.018                       | 0.894         | -0.055                      | 0.686       |
| L. postcentral gyrus–R. supramarginal gyrus| 0.105                       | 0.440         | -0.283                      | 0.034       |

DST, digit symbol test; L, left; NCT-A, number connection test type A; R, right.

Figure 1 Results of cerebral blood flow in patients with minimal hepatic encephalopathy. (a) Chosen regions of interest. (b) Original perfusion image of the region of interest. (c) Results of blood flow in a patient with minimal hepatic encephalopathy. [Color figure can be viewed at wileyonlinelibrary.com]
found within globus pallidus and basal ganglia of MHE patients after utilization of MRI. The toxic effects were suspected to be imposed by damaged excretion of bile, in spite of its uncertain significance. Furthermore, circulatory disturbances, which were possibly caused by portal systemic shunt and blocked removal of vasodilators (e.g. endotoxins and adenosine), also appeared as featured manifestations of MHE patients, and they could account for raised activity of sympathetic nerves. The MHE patients included in the present study mostly carried cirrhosis.

Among the MHE patients in this study, 15 brain FC alterations were found, including 12 weaker and 3

![Figure 2](image.png) Blood flow in the studied brain regions of patients with minimal hepatic encephalopathy (MHE) and control subjects. Blood flow in caudatum (a), globus pallidus (b), thalamus (c), and in putamen (d) in control and MHE patients.

**Table 4** Correlations between the neuropsychological test scores and mean cerebral blood flow in regions of interest (ROI) in patients with minimal hepatic encephalopathy

| ROI                  | NCT-A          | DST            |
|----------------------|----------------|----------------|
|                      | Correlation coefficient | P-value | Correlation coefficient | P-value |
| Left thalamus        | 0.014          | 0.921          | -0.048              | 0.728   |
| Right thalamus       | 0.019          | 0.892          | -0.055              | 0.689   |
| Left putamen         | 0.091          | 0.507          | -0.580              | 0.670   |
| Right putamen        | 0.391          | 0.024          | -0.358              | 0.017   |
| Left globus pallidus | 0.019          | 0.890          | -0.062              | 0.648   |
| Right globus pallidus| 0.012          | 0.933          | -0.051              | 0.709   |
| Left caudatum        | 0.009          | 0.945          | -0.058              | 0.673   |
| Right caudatum       | 0.017          | 0.902          | -0.062              | 0.648   |

DST, digit symbol test; NCT-A, number connection test type A.
significantly stronger alterations. Changes in brain FC were reported to be associated with neurocognitive functions and neuropathology mechanisms of MHE.\textsuperscript{26,27} Specifically, basal ganglia had multiple relationships with cerebral motor cortex, sensory cortex, limbic system, and fronto-orbital and fronto-lateral cortex.\textsuperscript{28} Thalamus, one key component of the basal ganglia-thalamo-cortical brain circuit, has been shown to play a role in filtering the sensory inputs from the cortex.\textsuperscript{29–31} In addition, dysfunction of anterior cingulate cortex and decreased connection with postcentral gyrus might lead to attention deficits and aberrant spontaneous neuronal activity, respectively.\textsuperscript{32–35} Abnormality of caudate might cause dysfunction of action consequence evaluation and sensory information transmission.\textsuperscript{36} Lobus insularis and parahippocampal gyrus are related with regulation of emotion and memory storage, respectively.\textsuperscript{37,38} In general, connectivity changes could help us infer particularized cognitive disorders of MHE patients, including attention, vigilance, psychomotor, and integrative dysfunctions.\textsuperscript{30,31}

![Receiver operating characteristic curve analysis of regional cerebral blood flow for diagnosis of minimal hepatic encephalopathy. (a) Right thalamus. (b) Right putamen. (c) Left caudatum. (d) Right globus pallidus. [Color figure can be viewed at wileyonlinelibrary.com]](image)

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Blood flows of specific brain regions in MHE patients were different from those in normal individuals, suggesting the potentially critical role of CBF in MHE development. Changes of CBF have been measured by single photon emission computed tomography (SPECT), positron emission tomography, dynamic susceptibility contrast (DSC)-enhanced perfusion MRI, and fMRI. Dynamic susceptibility contrast MRI is reportedly superior to SPECT for its improved spatial and temporal resolution, non-invasiveness, reduced costs, and acquisition of ample anatomical information. Although DSC-MRI could detect potential changes occurring during the early stages of MHE due to its close association with microcirculation (i.e., blood flow within capillaries), the technique necessitates injection of contrast agents, which could bring about trauma. Utilization of ASL could avoid this situation with the use of endogenous tracer; in other words, running blood is labelled for perfusion imaging. In this study, we applied ASL-fMRI to detect CBF and found that CBF was significantly increased in four ROIs, namely, the caudatum, globus pallidus, thalamus, and putamen, within MHE patients. The CBF values of MHE patients detected by SPECT and DSC-MRI were generally similar to ASL-fMRI, yet certain discrepancies were present. For instance, elevated CBF was reported in basal ganglia and left globus pallidus after use of DSC-MRI, whereas SPECT showed high perfusion in the mesial temporal lobe and hypoperfusion in the cortex among MHE patients. The inconsistency could be explained by differences of included individuals and perfusion techniques, among which ASL should be prioritized for not harming hepatic functions.

Concerning the correlation between CBF values and neuropsychological outcomes, aberrant CBF levels usually displayed pronounced associations with altered memory and intellectual functions. Particularly, reduced CBF may forecast decreased capacities of reasoning, confabulation, and message handling. Apart from changes in CBF amount, whether hemispheric CBF was initially asymmetric also remarkably affected late-stage memory performance and confabulatory behavior, which was explained by the hypothesis that subjects with former brain damage, one cause of interhemispheric asymmetry, would suffer from milder brain damage than those with normal brains. Even among normal subjects, notable asymmetry of CBF appeared to anticipate better performance in counting cubes. Interestingly, the current study failed to correlate CBF in basal ganglia with neuropsychological tests, yet a negative correlation was found in a report by Catafau et al. Use of a different battery of neuropsychological score tests may explain the above contrast. Previously, numerous neuropsychological tests validated for diagnosis of MHE were based on paper-pencil tests, including PHES and the repeatable battery for assessment of neurological status. The virtue of PHES lied in its commonly recognized high credibility, and it has emerged as the gold standard, as announced by the World Congress of Gastroenterology. As undertaking the whole PHES is time-consuming, it was later recommended to combine any two of the four tests (i.e. NCI-A, NCI-B, DST, and block design test) as the diagnostic criteria. However, this management made assessment of subjects’ concentration, visuomotor, psychomotor, and visuospatial capacities less comprehensive. Furthermore, the difficulty of paper-pencil tests to be interpreted necessitated several professionals to participate in the project. Finally, performance of paper-pencil tests failed to explain intracephalic changes. In contrast, CBF’s demands of technique and time are largely reduced, in that all it requires is fMRI. Moreover, intracephalic changes could be comprehended, and impaired tissues might be identified for specific parts of the brain through observation of hepatic imaging. Therefore, we believed that CBF could serve as a desirable biomarker for MHE diagnosis.

In conclusion, impairment of the connectivities resulting from the ganglia-thalamo-cortical circuit may play important roles in mediating the neurocognitive dysfunction of MHE patients, and the increased BF in basal ganglia, thalamus, and right putamen could be the potential biomarker for MHE detection. However, several limitations existed in our studies. In particular, the small sample size might affect the validity of our study. Further studies are needed to clarify the mechanisms by which the FC alterations affect MHE patients.

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