Imaging of Cerebrovascular Function in Chronic Traumatic Brain Injury

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Abstract

Traumatic cerebrovascular injury (TCVI) is a common pathologic mechanism of traumatic brain injury (TBI) and presents an attractive target for intervention. The aims of this study were to assess cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) using magnetic resonance imaging (MRI) to assess their value as biomarkers of TCVI in chronic TBI, characterize the spatial distribution of TCVI, and assess the relationships between each biomarker and neuropsychological and clinical assessments. Forty-two subjects (27 chronic TBI, 15 age- and gender-matched healthy volunteers) were studied cross-sectionally. CBF was measured by arterial spin labeling and CVR by assessing the MRI-blood oxygen level-dependent signal with hypercapnia challenge. A focused neuropsychological battery adapted from the TBI Common Data Elements and neurobehavioral symptom questionnaires were administered at the time of the imaging session. Chronic TBI subjects showed a significant reduction in mean global, gray matter (GM), and white matter (WM) CVR, compared with healthy volunteers ($p < 0.001$). Mean GM CVR had the greatest effect size (Cohen's $d = 0.9$). CVR maps in chronic TBI subjects showed patchy, multifocal CVR deficits. CBF discriminated poorly between TBI subjects and healthy volunteers and did not correlate with CVR. Mean global CVR correlated best with chronic neurobehavioral symptoms among TBI subjects. Global, GM, and WM CVR are reliable and potentially useful biomarkers of TCVI in the chronic stage after moderate-to-severe TBI. CBF is less useful as biomarker of TCVI. CVR correlates best with chronic TBI symptoms. CVR has potential as a predictive and pharmacodynamic biomarker for interventions targeting TCVI.

Keywords: cerebral blood flow; cerebrovascular reactivity; hypercapnia; magnetic resonance imaging-blood oxygen level dependent; neurovascular unit; traumatic brain injury; traumatic cerebrovascular injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a major cause of death and disability. In the United States, of approximately 2.5 million annual TBI emergency department visits for TBI, 52,000 patients die acutely and 92,000 survive with chronic disability.¹ Because survivors often live for many years, an estimated 5.3 million Americans live with TBI-related disability.^{2–5} The magnitude of this problem has led to extensive research and clinical trials of therapies to improve functional outcome.⁶ Unfortunately, all phase III human clinical trials to date have failed.^{7–10} Consensus conferences have concluded that imaging and biochemical biomarkers are

critical for the development of effective TBI therapies targeting injury-specific pathophysiology.^{6,11}

While the mechanisms responsible for TBI-related functional deficits are incompletely understood, substantial data indicate that traumatic cerebrovascular injury (TCVI) contributes to TBI-related disability. Because of the extensive plasticity of the cerebral vasculature, TCVI is an attractive target for intervention. Multiple well-established pharmacologic and non-pharmacologic therapies which promote blood vessel repair are currently available (e.g., statins, omega-3 fatty acids, phosphodiesterase-5 inhibitors, aerobic exercise) and may prove beneficial to appropriately selected TBI patients.^{12–14}

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TCVI is universally detected in experimental TBI^{15–19} and can result from both primary and secondary injury.^{20–25} Persistent microcirculatory deficits correlate with behavioral and cognitive deficits.¹⁷ In humans, cerebral blood flow (CBF) in acute TBI^{26–28} primarily shows focal or multifocal reduction. It is unclear whether decreased CBF reflects a primary vascular injury or a compensatory response to a primary neuronal injury with reduced metabolic demand. Cerebrovascular reactivity (CVR) assessed after a stimulus that does not affect neural activity represents an alternative assessment of TCVI. Established methods exist to study CVR in humans, including transcranial Doppler (TCD) or near infra-red spectroscopy (NIRS) with hypercapnia,^{29,30} but these tools have limited spatial resolution. In the first week after a bout, CVR is decreased in professional boxers and correlates with cumulative lifetime concussions.³¹ A meta-analysis of CVR after sports concussion concluded that CVR is decreased acutely.³² Since TCD and NIRS have poor spatial resolution, little is known about the focal, multifocal, or diffuse nature of TCVI with these modalities.

CVR also can be measured with magnetic resonance imaging (MRI) via the MRI-blood oxygen level-dependent (MRI-BOLD) sequences in response to a hypercapnia challenge and provide excellent spatial resolution.^{33–35} Mutch and colleagues examined CVR in adolescents with chronic post-concussive syndrome (PCS) after sports concussion and age-matched healthy controls with MRI-BOLD and found decreased mean CVR in the PCS group.³⁶ In this study, we measured CBF and CVR in chronic moderate-severe TBI subjects at least 6 months after injury. Additionally, we investigated how neurobehavioral symptoms and cognitive function correlate with CVR deficits. We hypothesize that CVR is decreased in chronic TBI, correlates with CBF, and is a reliable biomarker of TCVI.

Methods

Subjects

Adults between ages 18 and 55 years were consented and enrolled under an institutional review board-approved protocol (ClinicalTrials.gov NCT01762475). Inclusion criteria for TBI subjects included TBI 6 months to 10 years prior with Glasgow Coma Score between 3 and 12, post-traumatic amnesia >24 h, or TBI-related neuroimaging abnormality (computed tomography [CT] or MRI). Exclusionary criteria included any penetrating TBI, pre-existing disabling neurologic or psychiatric disorder, pregnancy, or unstable pulmonary or vascular disorder. Gender- and age-matched individuals with no history of TBI, pre-existing disabling neurological or psychiatric disorders, or pregnancy were enrolled as healthy controls (HC).

MRI imaging

MRI was performed on a Siemens Biograph mMR, fully integrated 3 Tesla MRI/PET. T1 Magnetization Prepared Rapid Gradient Echo (MPRAGE), susceptibility weighted imaging (SWI), T2- gradient echo sequences, diffusion weighted imaging, Diffusion tensor imaging (DTI), fluid-attenuated inversion recovery (FLAIR), pulsed arterial spin labeling (ASL) and MRI-BOLD sequences were acquired on each subject.

FLAIR images were acquired with the following parameters: repetition time/echo time (TR/TE) = 9090/112 msec, inversion time (TI) = 2450 msec, slice thickness = 3.0 mm. ASL images were acquired using a pulsed sequence with the PICORE Q2TIPS labeling scheme. The following parameters were established for the ac-

quisition of 111 2D-EPI volumes: TR/TE = 2700/15 msec, TI1/TI2 = 700/1800 msec.

Each participant underwent MRI-BOLD sequences with CVR hypercapnia measurements according to the methods developed previously.^{37,38} Short CO₂ challenges are similar to longer challenges in healthy volunteers,³⁷ and while shorter challenges have not yet been studied in TBI, the shorter challenge is able to reliably detect CVR deficit in a chronic neurodegenerative condition, Alzheimer disease.³⁸ Hypercapnia was induced via a Douglas bag alternating between a flow of room air and 5% CO₂ mixed with room air every minute for 7 min total while the MRI-BOLD images were acquired. End-tidal CO₂ (EtCO₂) was measured continuously using a capnograph (Smith Medical, Model 9004). These conditions (5% CO₂ for no longer than 1 min at a time) did not result in significant changes in mean arterial blood pressure, heart rate or oxygen saturation.³⁹ The sequence's parameters were TR/TE = 2,000/25 msec, flip angle = 80°, field of view = 220 × 220 mm², matrix = 64 × 64, 36 slices, thickness = 3.6 mm, no gap between slices, 210 volumes.

Data processing

ASL. Image data analyses were performed with the Statistical Parametric Mapping software (SPM12) and ASL data processing toolbox. The control and label ASL images were realigned and resliced to correct for head motion. They were smoothed using an isotropic Gaussian kernel with a 4 mm full-width at half-maximum. CBF images were reconstructed using the ASL Data Processing Toolbox: ASLtbx.

CVR. Voxel-by-voxel CVR maps were calculated based on a linear regression between BOLD signal time courses and the vascular input function. At baseline, the mean (standard deviation [SD]) EtCO₂ value is 39.9 (1.6) mm Hg and rise to 49 (1.2) mm Hg. The BOLD response signal is delayed from the EtCO₂ (Fig. 1) which corresponds to the time needed for the blood to travel between the lungs to the cerebrovascular network. The EtCO₂ time delay is individually corrected to achieve the maximum correlation between the two measures. We processed CVR data using a general linear model, with the EtCO₂ time course as the regressor to generate CVR maps (in the units of % BOLD change/mm Hg CO₂ change, %/mm Hg) for each subject. Images were spatially realigned and re-sliced to correct for head motion with SPM (realign and re-slice toolbox). We monitored six parameters (x, y, z translation and pitch, roll and yaw) to assure correct coregistration. The full method is previously described by Lu and colleagues.⁴⁰

The CVR, CBF, and FLAIR images were each co-registered with the MPRAGE images using SPM12. The CBF and CVR individual voxel intensity images were transformed into histograms, characterized by the mean and median. The individual images (CBF and CVR) were also segmented into white/gray matter and CSF. CVR and CBF maps were normalized into a Montreal Neurological Institute (MNI) brain template for group comparisons. For each patient, we computed a z-score map to characterize individual-level regional abnormalities. We used the normalized images to perform a voxel-by-voxel statistical parametric analysis to evaluate local abnormalities in individual's CBF and CVR, compared with a pool of HC. We define an abnormal voxel as any voxel with a z-score smaller than a specified threshold and belonging to a cluster size of at least 20 voxels. An abnormal voxel belongs to a specific cluster if one of its 26 neighbors is also an abnormal voxel.

Neurobehavioral assessments

A psychometrist administered a battery of neuropsychological tests and neurobehavioral symptom surveys selected from the TBI

FIG. 1. Time course of end-tidal CO₂ (EtCO₂) and blood oxygen level–dependent (BOLD) signal in the thalamus and average among the 15 healthy controls. The BOLD signal follows the EtCO₂ data with a temporal delay of approximately 15 sec.

Common Data Elements. They included: learning portions of the California Verbal Learning Test (CVLT), Word Reading subtest of the Wide Range Achievement Test (WRAT), Trail Making Test (TMT) Parts A and B, Digit Symbol and Symbol Search subsets of the Wechsler Adult Intelligence Scale (WAIS-IV), the Brief Symptom Inventory (BSI), and the Rivermead Post Concussion Symptoms Questionnaire. Raw scores on the CVLT, WRAT, TMT, and WAIS-IV were normalized for age and education.

Statistical analysis

The statistical analysis was done under Matlab statistical toolbox, using the Kolmogorov-Smirnov test for standard normal distribution. If one dataset rejected the null hypothesis at 5% significance level, we performed a Spearman rank order correlation. Otherwise, we performed Pearson linear correlation. The correlation coefficient is associated with a *p* value testing the hypothesis of no correlation. The statistical analysis of independent samples was done with two independent *t*-tests when the samples were normally distributed and with Mann-Whitney U-test otherwise.

Results

Demographics and TBI characteristics

Forty-two (27 TBI and 15 HC) age and gender-matched subjects were consented, enrolled in the study and had data available for analysis (Table 1). They had a mean ($\pm$ SD) age of 37.7 ± 16 years (range, 20–55). TBI subjects were enrolled a median of 25 months post-injury. The majority of TBIs occurred as a result of traffic incidents. All but two subjects had TBI-related neuroimaging findings on initial cranial CT, with hematomas and contusions the most common abnormality. Two subjects with initially normal CT scans had structural abnormalities identified by the study MRI. All TBI subjects were hospitalized and stayed a median of 3 days in the intensive care unit, and more than half received inpatient or outpatient rehabilitation. At enrollment the median Glasgow Outcome Scale-Extended score was 7 (range, 4 to 8) and the majority (77%) had persistent symptoms that met *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for persistent post-concussive syndrome. On

structural MRI, 15 showed focal encephalomalacia with or without microhemorrhage, five focal microhemorrhage only, one chronic subdural, and six were normal with either no evidence of prior trauma or resolution of earlier CT-evident subdural or subarachnoid hemorrhages. Four subjects had volume loss or global atrophy. All subjects underwent MRI-BOLD with hypercapnia challenge without adverse effect.

Cerebrovascular reactivity and cerebral blood flow

A qualitative visual analysis of CVR and CBF spatial distributions was made for each patient. Figure 2 shows representative two brain slices from three different subjects: one HC (slices 2A-1 and 2A-2) and two TBI subjects (slices 2B-1, 2B-2, 2C-1 and 2C-2). Each slice is displayed with the three imaging modalities: FLAIR, CBF and CVR, each aligned with the respective MPRAGE image.

CBF and CVR measures were higher in the gray matter (GM) than the white matter (WM) in all subjects. The CBF and CVR maps showed symmetric distribution in HC. However, in TBI patients, we observed asymmetry in CBF and CVR maps corresponding to a multifocal pattern of deficits. Figure 2C-5 displays two areas with focal CVR deficit with a corresponding area of CBF deficit. Most regions of decreased CBF or CVR correspond to structurally normal appearing parenchyma. Further, CVR deficits do not typically correspond to regions of decreased CBF.

For quantitative analysis, each individual's mean and median CBF and CVR were measured in three compartments: whole-brain, GM, and WM. The distribution of CVR and CBF values are shown in Figure 3. CVR and CBF are normally distributed (D'Agostino Pearson test = 0.28 and 0.42). These parameters (mean and median) were averaged for the two groups, HC and TBI (Table 2).

For CVR, Table 2 summarizes the differences between the two groups with a significant decrease in TBI subjects (*p* value < 0.001); Cohen's *d* = 0.8, and a receiver operator characteristic (ROC) analysis showed an area under the curve (AUC) higher than 0.8 only for the mean and median values. However, for CBF, there was no

TABLE 1. DEMOGRAPHICS, TBI CHARACTERISTICS, NEUROPSYCHOLOGICAL TESTS AND NEUROBEHAVIORAL SYMPTOM SURVEY ANALYSIS

	TBI (n=27)	HC (n=15)	P
Age (years), mean $\pm$ SD	37.6 $\pm$ 15	38.1 $\pm$ 7.4	0.89
Gender, % male	70	79	
Education (years), median (IQR)	15 (13–16)	16 (14–20)	0.02
Time since TBI (months), median (IQR)	25 (17–34)	*	*
Road traffic incident (%)	59	*	*
LOC >30 min (%)	42	*	*
Abnormal neuroimaging, CT or MRI (%)	92	*	*
Days in ICU, median (IQR)	3 (1 to >14)	*	*
Received rehabilitation (%)	54	*	*
WRAT Standard	99.2 $\pm$ 10.3	103.5 $\pm$ 9.8	0.097
WAIS-PSI	101.8 $\pm$ 18.3	104.7 $\pm$ 15.3	0.299
TMT A	46.1 $\pm$ 14.7	50.8 $\pm$ 14.6	0.163
TMT B	63.6 $\pm$ 32.3	59.6 $\pm$ 22.5	0.338
CVLT	52.5 $\pm$ 12.7	52.1 $\pm$ 10.3	0.457
Rivermead 3	2.59 $\pm$ 1.8	0.27 $\pm$ 0.6	< 0.0001
Rivermead 13	16.15 $\pm$ 11.9	2.07 $\pm$ 2.7	< 0.0001
BSI Global	5.2 $\pm$ 6	2 $\pm$ 2.9	0.067
BSI Somatic	1.23 $\pm$ 1.48	0.133 $\pm$ 0.352	0.0076
BSI Depression	2.42 $\pm$ 3.67	1.07 $\pm$ 1.83	0.1898
BSI Anxiety	1.77 $\pm$ 2.21	0.8 $\pm$ 1.15	0.124

BSI global is defined as the sum of BSI somatic, BSI depression and anxiety. WRAT, WAIS, TMT-A, TMT-B and CVLT are normalized T scores.

TBI, traumatic brain injury; HC, healthy control; SD, standard deviation; IQR, interquartile range (25th and 75th); LOC, loss of consciousness; CT, computerized tomography; MRI, magnetic resonance imaging; ICU, intensive care unit; Inpt/Outpt, inpatient or outpatient; GOS-E, Glasgow Outcome Scale-Extended; WRAT, Wide Range Achievement Test; WAIS-PSI, Wechsler Adult Intelligence Scale-Processing Speed Index; TMT, Trail Making Test; CVLT, California Verbal Learning Test; BSI, Brief Symptom Inventory.

statistical difference between the two groups in mean, median, skewness, or kurtosis (p values >0.01, AUC <0.7, and Cohen's $d=0.7$).

All scans were normalized into the MNI space for a voxel-by-voxel statistical parametric analysis to evaluate multifocal abnormalities in individual CVR or CBF maps for each TBI subject compared to the mean HC. Figure 4A and 4B represent the voxels with a low CBF and CVR z-score. A z-score of -2.75 was selected as it maximized the AUC for discriminating between TBI and HC (AUC=0.82 for CVR and AUC=0.89 for CBF). From these maps, we counted the number of voxels in which the z-score value is lower than a given threshold. We found no voxels in which the CVR value was statistically higher in TBI subjects than in controls. We also found a median volume of abnormal CVR of 0.48 cm^3 (mean [SD] $2.82 [5.33 \text{ cm}^3]$) for the HC group and a median volume of 6.86 cm^3 (mean [SD] $15.3 [21.2 \text{ cm}^3]$) for the TBI group ($p=0.021$, AUC=0.87, effect size=0.73). The same analysis for CBF yielded a median volume of 0 cm^3 (mean [SD] $0.88 [2.44 \text{ cm}^3]$) for the HC group and a median volume of 7.50 cm^3 (mean [SD] $16.7 [28.2 \text{ cm}^3]$) for the TBI group ($p=0.07$, AUC=0.89, Cohen's $d=0.68$).

Another measure of the voxel-by-voxel statistical parametric analysis is the size and number of abnormal CVR or CBF voxel clusters. Each abnormal voxel was defined as belonging to a cluster if one of its 26 neighbors is an abnormal voxel. This definition gives us, for the HC CVR maps, a median cluster number of 1 (mean [SD] $1.20 [1.37]$) with a mean (SD) cluster volume of $0.30 (0.49) \text{ cm}^3$. For the TBI CVR maps, a median cluster number of 4 (mean [SD] $4.14 [3.30]$) with mean (SD) cluster volume of $0.46 (0.80) \text{ cm}^3$ (p value <0.01, AUC=0.75, effect size=0.79). Alternatively for the CBF analysis (again with a threshold z-score of -2.75), each HC subject has a median cluster number of 2 (mean [SD] $2.9 [1.8]$) with a mean (SD) cluster volume of $0.30 (1.33) \text{ mm}^3$, and each TBI subject has a median cluster number of 7 (mean [SD] $8.6 [5.5]$) with a mean (SD) volume of $1.94 (5.0) \text{ mm}^3$ (p value <0.001, AUC=0.84, effect size=0.84).

FIG. 2. Examples of cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) maps from one healthy volunteer (A) and two TBI patients (B and C) acquired with 3 magnetic resonance imaging (3 Tesla) sequences (fluid-attenuated inversion recovery [FLAIR], ASL and MRI-BOLD with hypercapnia challenge: FLAIR (A-1, A-2, B-1, B-2, C-1, C-2), CBF (A-3, A-4, B-3, B-4, C-3, C-4) and CVR (A-5, A-6, B-5, B-6, C-5, C-6). The white numbered circles indicate focal CBF and CVR deficits. The TBI patient in the middle three panels had no visible abnormalities on standard anatomical imaging, while the TBI patient in the last three panels had a lesion in the left frontal subcortical white matter (C-2). For example, the CVR map in B-5 displays three regions with focal CVR deficit, which are not evident in the CBF map (B-3). This mismatch can also be appreciated in B-6 and C-6. In C-6, we observed post-traumatic encephalomalacia in the corresponding FLAIR image (1C-2) and, in the vicinity of the lesion, we noted a decrease of CVR (C-6, region 2) but not of CBF (C-4).

FIG. 3. Mean distribution of cerebrovascular reactivity (CVR) values (top) and cerebral blood flow (CBF; bottom) for the healthy control (HC) and traumatic brain injury (TBI) population in the whole brain (union between the gray matter and the white matter). The HC histogram (blue) is the average of 15 patients and the TBI histogram (red) is the average of 27 patients. The error bar is the standard deviation of CVR (or CBF) for one group within a given bin. HC have a median CVR value of 0.173 ± 0.014 and TBI have a median CVR value of 0.140 ± 0.230 (p value < 0.001). HC have a median CBF value of 38.0 ± 12.8 and TBI a median CBF of 33.0 ± 8.1 (p value $= 0.04$).

To compare the two modalities (CBF and CVR), we measured the correlation between the mean whole brain CVR and the mean whole brain CBF, for all study subjects (Fig. 5). The two measures were not correlated for all subjects (Spearman's correlation $= 0.04$, $p = 0.7$), or within HC group (Spearman correlation $= 0.11$, $p = 0.7$) or within TBI group (Spearman's correlation $= 0.005$, $p = 0.98$).

Neurobehavioral assessments

Each subject's normalized scores for neuropsychological tests were calculated. Rivermead total and subcomponent scores were used for analysis. Table 1 shows the results for the HC and the TBI groups; the only significant differences between the two groups are seen with the WRAT reading test, and the Rivermead scores. The relationship between individual TBI neurobehavioral data with the individual TBI CVR data (global, GM, WM and z-score) was assessed (table 1). Only Rivermead 13 showed a trend for a corre-

TABLE 2. CHARACTERISTICS OF CVR AND CBF FOR HC AND TBI GROUP

	CVR (%BOLD/mm Hg)		CBF (mL/100 g/min)	
	mean	median	mean	median
Whole brain				
HC	0.207 ± 0.020	0.173 ± 0.014	37.5 ± 8.6	38.0 ± 12.8
TBI	0.173 ± 0.029	0.140 ± 0.23	37.7 ± 8.6	33.0 ± 8.1
Effect size	0.81	0.9	0.66	0.68
AUC	0.8	0.88	0.65	0.7
p value	< 0.001	< 0.00001	0.08	0.04
Gray matter				
HC	0.228 ± 0.022	0.191 ± 0.016	45.1 ± 11.1	43.9 ± 13.3
TBI	0.190 ± 0.031	0.154 ± 0.025	40.3 ± 7.4	37.9 ± 8.8
Effect size	0.81	0.9	0.66	0.68
AUC	0.79	0.89	0.66	0.7
p value	< 0.001	< 0.00001	0.07	0.03
White matter				
HC	0.158 ± 0.016	0.143 ± 0.012	33.1 ± 10.0	29.3 ± 11.3
TBI	0.136 ± 0.024	0.117 ± 0.021	29.88 ± 7.1	25.6 ± 7.7
Effect size	0.76	0.86	0.64	0.66
AUC	0.8	0.88	0.65	0.69
p value	< 0.001	< 0.00001	0.13	0.08

CVR, cerebrovascular reactivity; CBF, cerebral blood flow; HC, healthy control; TBI, traumatic brain injury; BOLD, blood oxygen level-dependent; AUC, area under the curve.

lation (correlation with the global CVR is 0.383 ($p = 0.049$). There was no correlation between normalized neuropsychological test or symptom survey scores and CBF.

Discussion

In this study, we used MRI to investigate the spatial distribution of TCVI in chronic moderate and severe TBI, and assessed the potential of CBF and CVR as biomarkers of TCVI. We assessed two vascular measures, a static measure of CBF via ASL sequences and a dynamic measure of CVR by MRI-BOLD with hypercapnia challenge. While nearly all TBI subjects had abnormalities detectable on structural imaging (MPRAGE, FLAIR, or SWI), most of the regions of low CBF or CVR appeared in normal-appearing brain regions (however, a DTI analysis can reveal focal axonal injury not detectable on structural imaging). CVR (and to a lesser extent CBF) maps show asymmetry in the chronic TBI population that reliably differentiates them from HC. The patchy, "moth-eaten" appearance of the CVR maps in the chronic TBI patients were distinct from the symmetrical distributions in the CBF and CVR maps of HC. In areas of visible encephalomalacia on structural MRI, we noted a decrease in both CBF and CVR. However, most regions with CVR deficit are not associated with an apparent change in CBF. We interpret this finding as indicating that in these regions the microvasculature has normal or near-normal density and therefore adequate blood flow, but has been sufficiently damaged so that it cannot vasodilate normally. Our findings support the conclusion that such physiologically impaired microvasculature distinguishes chronic TBI from HC subjects.

Within subjects, CBF and CVR measures poorly correlate with each other (Fig. 5). The TBI and HC subjects form two clusters separated by their CVR values (below or above 0.186 %BOLD/mm Hg). In chronic TBI we see a spectrum of local changes in cerebral vasculature. At one end of the spectrum, local CBF is completely

FIG. 4. Map of abnormal cerebrovascular reactivity (CVR) z-score values (color) overlay on the Magnetization Prepared Rapid Gradient Echo (MPRAGE; top) and map of abnormal cerebral blood flow (CBF) z-score values (color) overlay on the MPRAGE (bottom). The subject is a 51-year-old male with traumatic brain injury whose non normalized images are shown in Figure 2-B2 to 2-B4.

absent as a result of irreparably damaged vasculature and associated tissue loss. In this case, absent blood flow correlates with an absence of CVR. At the other end of the spectrum, local CBF is normal, but CVR measures are decreased. Here, we hypothesize that focal injury results in endothelial damage sufficient to impair local function, including vasodilation in response to stimuli.

In our study, CBF measures were not a sensitive biomarker of TCVI. Compared with the HC group, there was only a modest overall CBF decrease in the TBI group, either in the mean CBF globally or within the GM or WM compartments. However, the voxel-by-voxel statistical analysis did show a more robust difference in the number of CBF bad voxels between the two groups. After normalizing individual CBF measures and creating CBF maps, we saw that the CBF changes are multifocal.

Based on the ROC analysis, CVR is a sensitive biomarker of TCVI as a specific endophenotype that may explain part of the variability in outcome. In this study, all measures of CVR—global,

GM and WM—are significantly lower in TBI than HC subjects, with the mean global and GM measures having the greatest effect size. Creating CVR maps by spatially normalizing the CVR measures in MNI space provides information on the distribution of the microvascular injury in chronic TBI. The abnormal voxel values form clusters in the TBI groups which are larger in both number and size than in the HC group. These clusters are found throughout the brain and this metric is important for quantifying the injury independent of specific regions of interest. The cluster analysis also demonstrates the multifocal and stochastic distribution of TCVI.

There was no significant relationship between neuropsychological test performance in the TBI subjects and CBF or CVR measures, although there was a weak correlation between CVR and chronic TBI symptoms, especially parameters regarding neurobehavioral symptoms (fatigue, sleep disturbance, irritability, depressed mood, decreased frustration tolerance, poor concentration/memory, vision problems, and sensitivity to light or noise). The

FIG. 5. Individual measures of cerebrovascular reactivity (CVR; x-axis) versus cerebral blood flow (CBF; y-axis). The blue dots represent healthy controls (HC) and the red dots traumatic brain injury (TBI) subjects. The Spearman's correlation between CBF and CVR values is 0.04 ($p=0.7$).

relationship between CVR and chronic TBI symptoms suggest a potential physiologic basis for that group of symptoms that possibly would be responsive to TCVI-directed therapies. It also suggests that global measures of CVR may be useful predictive and/or pharmacodynamic biomarkers of TCVI-targeted therapies used to ameliorate chronic TBI symptoms.

It is not surprising that the relationship between global CBF and CVR with neurobehavioral symptoms and neuropsychological performance is weak or absent. None of the currently available structural or functional neuroimaging biomarkers are highly correlated with neuropsychological functioning, or symptom self-report. It is likely that analysis of regional abnormalities in neuroimaging parameters will be needed to identify relationships with particular cognitive functions. It is likely that large sample sizes, multiple biomarkers, and sophisticated multivariate models will be required to explain a substantial fraction of the variance in outcome after TBI. We anticipate that CVR will be among the biomarkers that will ultimately be incorporated into such models.

In summary, this study shows that CVR, a dynamic measure of microvascular function, is a sensitive biomarker of TCVI in chronic moderate/severe TBI, with the global and GM CVR measures the most sensitive for distinguishing TBI from HC. It shows promise as an adjunct tool in the diagnostic assessment of TBI and may potentially be useful as a pharmacodynamic biomarker of vascular-directed therapies.⁴¹ Further, CVR changes after moderate-severe TBI are not uniform throughout the brain, but rather show a patchy, multifocal, and stochastic distribution, which are poorly correlated to regions of low CBF or encephalomalacia on structural MRI.

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