Perfusion deficits in patients with mild traumatic brain injury characterized by dynamic susceptibility contrast MRI

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Perfusion deficits in patients with mild traumatic brain injury (TBI) from a military population were characterized by dynamic susceptibility contrast perfusion imaging. Relative cerebral blood flow (rCBF) was calculated by a model-independent deconvolution approach from the tracer concentration curves following a bolus injection of gadolinium diethylenetriaminepentaacetate (Gd-DTPA) using both manually and automatically selected arterial input functions (AIFs). Linear regression analysis of the mean values of rCBF from selected regions of interest showed a very good agreement between the two approaches, with a regression coefficient of $R = 0.88$ and a slope of 0.88. The Bland–Altman plot also illustrated the good agreement between the two approaches, with a mean difference of $0.6 \pm 12.4 \text{mL/100 g/min}$. Voxelwise analysis of rCBF maps from both approaches demonstrated multiple clusters of decreased perfusion ($p < 0.01$) in the cerebellum, cuneus, cingulate and temporo-parietal gyrus in the group with mild TBI relative to the controls. MRI perfusion deficits in the cerebellum and anterior cingulate also correlated ($p < 0.01$) with neurocognitive results, including the mean reaction time in the Automated Neuropsychological Assessment Metrics and commission error and detection T-scores in the Continuous Performance Test, as well as neurobehavioral scores in the Post-traumatic Stress Disorder Checklist–Civilian Version. In conclusion, rCBF calculated using AIFs selected from an automated approach demonstrated a good agreement with the corresponding results using manually selected AIFs. Group analysis of patients with mild TBI from a military population demonstrated scattered perfusion deficits, which showed significant correlations with measures of verbal memory, speed of reaction time and self-report of stress symptoms. Published 2013. This article is a U.S. Government work and is in the public domain in the USA.

Keywords: dynamic susceptibility-weighted MRI; perfusion; arterial input function; relative cerebral blood flow; traumatic brain injury

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of death and disability in young people at the most productive time of their lives (1). Each year an estimated 1.5 million people in the USA alone sustain nonfatal TBI (2). Approximately 80% of these injuries are classified as mild TBI (3). Largely as a result of injuries sustained in conflicts in Iraq and Afghanistan, military estimates...
of TBI in deployed personnel indicate that between 10% and 20% may have suffered a mild TBI during deployment (4). Mild TBI accounted for 44% of the patients treated at Walter Reed Army Medical Center (WRAMC) for combat-related TBI (5). The American Congress of Rehabilitation Medicine (ACRM) criteria define mild TBI as a mechanically induced physiological disruption of brain function manifested by any one of the following: a loss of consciousness (LOC), a loss of memory for events immediately preceding or following the injury, an alteration in mental status at the time of injury or focal neurological signs that may or may not be transient (6). A variety of symptoms are common in the acute phase and can include physical symptoms, such as dizziness or headache, cognitive complaints, such as slowed processing, or emotional complaints, such as irritability (7). The common course of recovery in mild TBI is improvement over days and weeks, with substantial improvement by 1 month post-injury, although symptom reporting continues to be experienced by a substantial number of subjects with TBI of all severity levels at 1 year post-injury (8).

In a military population, persistent post-concussive symptoms have been linked to emotional distress, including post-traumatic stress disorder (PTSD) (9–11). In acute military mild TBI (as in civilian mild TBI), neuropsychological deficits are evident in the first few days following injury (12). Neuropsychological deficits are not typical, however, beyond this very acute period. Belanger et al. (13), in a meta-analysis, found that the overall effect of mild TBI on neuropsychological functioning was moderate (d = 0.54). However, in unselected or prospective samples, the overall analysis revealed no residual neuropsychological impairment by 3 months post-injury. In the sports concussion literature, no residual neuropsychological effects are apparent when neuropsychological testing is completed more than 7 days post-injury (14). There may be subtle, but measurable, changes in cognitive functioning associated with the combat experience itself (15). However, neuropsychological and functional deficits appear to be related more to combat-associated depression and PTSD than to mild TBI (16).

Most patients with mild TBI have negative computed tomography (CT) or conventional MRI (17). Several studies suggest that functional neuroimaging employing 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) and perfusion single-photon emission computed tomography (SPECT) may be more sensitive than conventional imaging modalities for the evaluation of TBI (18–21). However, the results of PET studies conducted in patients with mild TBI are inconsistent (22–24). Although most SPECT studies have revealed hypoperfusion in patients with mild TBI (25–27), these findings failed to explain the severity of the clinical symptoms and the relationship between SPECT findings and neuropsychological testing (28). There remains significant desire in the military and scientific community for ‘biomarkers’ of TBI (29). Advanced imaging techniques hold promise for a better understanding of who has suffered a mild TBI, the nature of persistent symptoms and the prognosis over functioning (30–32).

Perfusion measurement using MR techniques can be divided into two domains: the slightly invasive domain with the use of external contrast media and the noninvasive domain based on magnetic labeling of inherent blood properties. Dynamic susceptibility contrast (DSC) MR perfusion imaging allows the assessment of cerebral hemodynamics by estimation of the tissue concentration–time curves after bolus injection of intravascular contrast agents (33). Following the pioneering work by Ostergaard et al. (34,35), relative cerebral blood flow (rCBF) estimation using DSC imaging has become feasible on clinical scanners (36) and has demonstrated early promise in the evaluation of patients in the clinical setting (37,38). Arterial spin labeling (ASL) falls into the second category, which is entirely independent of the use of a contrast agent (39,40). However, it offers a low signal-to-noise ratio and lower spatial resolution compared with DSC imaging. Furthermore, DSC imaging is well established and readily available in most hospitals, whereas ASL is still regarded as a research tool and is not widely available.

The most common approach to the determination of rCBF from tissue concentration–time data in DSC imaging, in theory, requires the deconvolution of an arterial input function (AIF), i.e. the tracer concentration throughout the measurement period in the vessels feeding the tissue of interest (34). Ideally, a unique AIF to each voxel should be identified. Because this is not practical in the clinical setting, most analysis methods assume that AIF is uniform across the brain, and therefore apply a single AIF to the entire brain. Because of this assumption, the determination of an accurate and representative AIF is of critical importance for the calculation of rCBF (41). Typically, AIF is obtained directly from MR images by selecting a small number of voxels containing one of the principal arterial vessels. AIF must satisfy several criteria related to the characteristics of the arteries: early arrival time of the injected bolus, a large peak and a rapid transit time as characterized by a narrow full width at half-maximum (FWHM). The pixel selection can be made manually or by means of automatic algorithms. Manual selection presents some limitations, deriving mainly from the human component in the procedure. A trained operator selects the arterial voxels on the basis of his/her experience and judgment, which reduces the procedure's reproducibility. Moreover, vessels are difficult to locate because of the partial volume effects presented in the low-spatial-resolution DSC data. The current approaches for automated AIF selection can be classified into three main categories: (i) curve fitting to a model function and choice of AIF candidates based on the fitted parameters (42,43); (ii) use of cluster analysis to assign each concentration curve into the most possible tissue class based on multiple features (38); or (iii) combination of both curve fitting and cluster analysis (44,45). Carroll et al. (46) presented a simple method on the basis of the arrival time of contrast medium and integrated signal change of the voxel. Nevertheless, their approach picks a single best candidate that can be subjected to noise contamination. Butman and Tasicyn (47) developed a fully automated AIF selection approach by the application of successive filters based on the standard deviation (SD) maps of the DSC data. A reliable AIF was produced by a small population of ‘best candidate voxels’ rather than a single optimum candidate voxel.

The study presented in this article seeks to evaluate and apply the work of Butman and Tasicyn (47) for automated AIF selection to the characterization of cerebral perfusion in patients with combat-related mild TBI. We hypothesize that the proposed cerebral perfusion analysis will be able to identify abnormalities in rCBF resulting from mild TBI and the abnormal blood flow will correlate with neuropsychological measurements. In this article, rCBF values calculated by model-independent deconvolution of the tracer concentration curve from automatically selected AIFs were compared with those calculated from AIFs selected manually from both middle cerebral arteries (MCAs). Furthermore, a preliminary examination of correlations between perfusion deficits and neuropsychological functions was assessed to investigate the correlation between hypoperfusion and functional impairment in military patients with mild TBI.
METHODS

Patient selection
Twenty-seven active-duty military individuals returning from Afghanistan or Iraq with a clinical diagnosis of mild TBI were selected for the study. Subjects were seen at WRAMC. The diagnosis of TBI was based on a routine comprehensive clinical evaluation undertaken by medical/healthcare professionals at WRAMC. As part of the standard clinical pathway, all patients treated at WRAMC, who are considered to be ‘at risk’ for TBI, undertake a TBI evaluation. A low threshold is purposely used to classify patients ‘at risk’ for TBI. Typically, patients are considered ‘at risk’ for TBI if they sustained an injury to any part of their body above the shoulders during a battle- or non-battle-related event, or were injured in any way by an event such as a blast, assault, motor vehicle accident or fall. For the large majority of patients, these evaluations are completed by a physician’s assistant who is trained to evaluate the presence of TBI. In some cases, evaluations are also completed by other healthcare professionals, such as neuropsychologists, social workers and nurses who are trained to evaluate TBI. Evaluations typically include: (i) a patient interview; (ii) a medical chart review including the review of in-theater medical records when available; (iii) case conferencing; and (iv) family interview and gathering of other collateral information (if available). The diagnosis and severity of TBI is based on the presence and duration of LOC, presence and duration of post-traumatic amnesia (PTA), presence and duration of alteration of consciousness (AOC), neurolological scan results and Glasgow Coma Scale scores (if available). Self-reported symptoms are routinely obtained during the TBI evaluation, but are not used for diagnostic or classification purposes. Mild TBI was defined as a head trauma-induced LOC, change in mental status (e.g. confusion or disorientation) and/or PTA, and the absence of intracranial abnormalities on CT or structural MRI scans undertaken within the first few days and/or weeks post-injury. The duration of PTA was less than 24 h and the duration of LOC was less than 15 min. Subjects were not excluded if they exhibited stress symptoms or had extracranial injuries.

In addition, 11 age-matched healthy individuals eligible for the Defense Enrollment Eligibility Reporting System (DEERS) were recruited as controls. Patients were instructed not to take any sedative- or psychoactive medications that could interfere with functional MRI (fMRI) scanning. All participants received a comprehensive MRI examination with DSC perfusion imaging performed towards the end of the examination. Sixteen of the 27 patients also underwent neuropsychological assessment. Table 1 summarizes the demographics of the participating subjects. The mean age of the patients was 25.9 ± 4.2 years and the mean age of the control subjects was 25.9 ± 6.6 years. The time since injury of the patients was 113 ± 74 days. Of the 27 TBI patients, 14 had blast-related TBI and 13 were a result of other causes. The majority of the patients experienced a brief LOC lasting a few seconds to 2 min, with one patient experiencing LOC of approximately 15 min. Four of the patients experienced PTA of less than 5 min. Table 2 lists the most common medications that were prescribed to more than three patients at the time of MRI.

Data acquisition

Image acquisition

The comprehensive MR protocol included anatomical imaging, four paradigms of fMRI, diffusion tensor imaging (DTI), susceptibility-weighted imaging (SWI), spectroscopic imaging and DSC perfusion imaging. Images were acquired on a 3T whole-body scanner (Discovery MR750; GE Healthcare, Milwaukee, WI, USA) equipped with a 32-channel phased array head coil (MR Instruments, Inc., Minnetonka, MI, USA). T1-weighted structural images were acquired with a three-dimensional BRAVO sequence: TR/TE = 6.7/2.5 ms; flip angle, 12°; resolution, 1 × 1 × 1 mm³. DSC images were acquired following an injection of 20 mL of gadolinium diethylenetriaminepentaacetaetate (Gd-DTPA) administered at a rate of 5 mL/s using a two-dimensional gradient-echo, echo-planar imaging sequence with the following parameters: TR/TE = 2800/22.3 ms; flip angle, 60°; resolution, 2 × 2 × 4 mm³; 36 slices for whole-brain coverage. For each whole-brain volume acquisition, 40 sequential images were recorded at intervals equal to the repetition time (2800 ms).

Neuropsychological assessment

Patients were administered a battery of neuropsychological tests for the evaluation of neurobehavioral symptoms and neurocognitive

Table 1. Demographics of all participants (median values are presented in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control</th>
<th>p</th>
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<tbody>
<tr>
<td>Number</td>
<td>27</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>3</td>
<td>0.24</td>
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<tr>
<td>Age (years)</td>
<td>25.9 ± 4.2 (27.1)</td>
<td>25.9 ± 6.6 (23.7)</td>
<td>0.99</td>
</tr>
<tr>
<td>Time of injury (days)</td>
<td>113 ± 74 (89)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Blast injury</td>
<td>14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nonblast injury</td>
<td>13</td>
<td>–</td>
<td>–</td>
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Table 2. Patient medication summary

<table>
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<tr>
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<th>Drug effects</th>
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<tr>
<td>Docusate sodium</td>
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<td>Laxative</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>9</td>
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<tr>
<td>Gabapentin</td>
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<td>Anticonvulsant</td>
</tr>
<tr>
<td>Hydrocodone</td>
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<tr>
<td>acetaminophen</td>
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<td></td>
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<tr>
<td>Doxycycline</td>
<td>6</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Prednisone</td>
<td>6</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Norrixitryline hcl</td>
<td>6</td>
<td>Antidepressant</td>
</tr>
<tr>
<td>Sennosides</td>
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</tr>
<tr>
<td>Celcoxib</td>
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</tr>
<tr>
<td>Lidoicaine</td>
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<tr>
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<td>Pain reliever</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
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<tr>
<td>Promethazine hcl</td>
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<td>Antihistamine</td>
</tr>
<tr>
<td>Quetiapine fumarate</td>
<td>4</td>
<td>Antipsychotic</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>3</td>
<td>Anticonvulsant</td>
</tr>
<tr>
<td>Diphenhydramine hcl</td>
<td>3</td>
<td>Antihistamine</td>
</tr>
<tr>
<td>Ondansetron hcl</td>
<td>3</td>
<td>Antiemetic</td>
</tr>
<tr>
<td>Zolpidem tartrate</td>
<td>3</td>
<td>Sedative</td>
</tr>
</tbody>
</table>
functioning. The battery consisted of the Verbal Fluency subtests from the Delis–Kaplan Executive Function System (DKEFS), the Conners’ Continuous Performance Test (CPT), the California Learning Test-Second Edition (CVLT-II), selected subsets from the Wechsler Adult Intelligence Scale (WAIS-III), the Wechsler Test of Adult Reading (WTAR), the Personality Assessment Inventory (PAI), the 36-item Short Form Health Survey (SF-36), the Neurobehavioral Symptom Inventory (NSI), the Post-traumatic Stress Disorder Checklist-Civilian Version (PCL-C), the Test of Memory Malingering (TOMM) and the Automated Neuropsychological Assessment Metrics (ANAM). The tests were administered by trained psychologists familiar with the needs of the study and the necessity for valid and reliable assessments.

Data analysis

Calculation of CBF

All MRI data were anonymized prior to analysis with AFNI software (48) (http://afni.nimh.nih.gov/afni). The perfusion images were first assessed for the presence of gross head motion and contrast enhancement prior to further analysis. Two patients and one control subject were excluded from further data analysis as a result of gross motion. In addition, data from one control subject showed no visible enhancement and were also discarded.

After slice timing correction, the signal intensity changes were converted into concentration–time curves assuming a linear relationship (49) between the paramagnetic contrast agent concentration and the change in the transverse relaxation rate \( R(t) \):

\[
C(t) = \Delta R(t) = -\log \left( \frac{S(t)}{S_0} \right) / TE
\]

where \( C(t) \) is the estimated tracer concentration, \( t \) is the time, \( S_0 \) is the average baseline signal before injection of the tracer, \( S(t) \) is the tissue signal intensity at time \( t \) and \( TE \) is the echo time.

According to the central volume theorem (50), the concentration \( C(t) \) of tracer at time \( t \) within a given voxel of interest can be written as:

\[
C(t) = CBF \int_0^t C_a(\tau) R(t-\tau) d\tau
\]

where \( C_a(\tau) \) is AIF and CBF is the cerebral blood flow. \( R(t) \) is the residue function describing the fraction of injected tracer still present in the vasculature at time \( t \):

\[
R(t) = \left[ 1 - \int_0^t h(\tau) d\tau \right]
\]

As \( h(\tau) \) is a probability density function, \( R(0) = 1 \) and \( R(t) \) is a positive decreasing function of time.

From Equation [2], the initial height of the deconvolved concentration–time curve corresponds to CBF. To calculate CBF, a model-independent deconvolution with regularization was performed with the 3dtfit function in AFNI using both automated and manually selected AIF.

Automated and manual AIF selection

The automated AIF selection was performed with an in-house software (47). The initial AIF candidate map was estimated by calculating two SD maps: the baseline map (SDb) and the baseline plus initial rise (3–6 s or one to two time points after the start of the tracer injection) map (SDa):

\[
SD_b = SD[1 \ldots t_0] \\
SD_a = SD[1 \ldots t_{0+1}] \text{ or } SD[1 \ldots t_{0+2}]
\]

where \( t_0 \) is the bolus arrival time index. \( t_0 \) was identified as the time at which the intensity difference between two consecutive points on the mean concentration curve of the whole brain was greater than 1.5% of the average baseline intensity. As shown in Figure 1a, the SDa map showed signal in the expected locations of the vasculature as well as in voxels that had a high baseline fluctuation. Subtracting SDb (Fig. 1b) from SDa eliminated those voxels with high baseline fluctuation, yielding a map selective for arterial voxels (Fig. 1c). Adjusting the threshold reduced the number of candidate voxels to the user-specified target number (Fig. 1d). The candidate voxels were further refined using the exclusion criteria outlined below:

- voxels having maximum values 10 s before or after the contrast arrival time;
- voxels having negative concentration with an absolute value of more than one-half of its maximum;
- voxels having FWHMs larger than the mean FWHM of the brain;
- voxels having a time to peak (TTP) larger than the mean TTP of the brain.

For comparison, two AIF candidate voxels satisfying characteristic criteria (large peak, early arrival and narrow FWHM) were

Figure 1. (a) SDa map: standard deviation map of baseline plus the initial rise after bolus arrival (3–6 s). (b) SDb map: standard deviation map of the baseline only. (c) Subtraction map of SDa – SDb, (d) Arterial input function (AIF) candidates generated by thresholding (c).
also manually selected from the right and left MCA using the AFNI graphic user interface. In both manual and automated approaches, the mean arterial curve was created by averaging across the concentration curves of each of the candidates.

Registration and segmentation

Nonbrain voxels were removed from the $T_1$ images using AFNI’s 3dautomask function. Each brain volume was inspected section by section and mis-segmentations were adjusted manually using the AFNI graphic user interface. Correction for image distortion resulting from echo-planar image acquisition was accomplished using a nonlinear deformation algorithm from the Advanced Normalization Tools (ANTS) to align the DSC images to the structural $T_1$ images (26) (http://picsl.upenn.edu/ANTS). The structural images were subsequently warped to a population-based template using ANTS. A white matter mask for the three-dimensional $T_1$ template was generated using the automated segmentation tool in FMRIB-FSL software (51) (http://www.fmrib.ox.ac.uk/fsl/) for rCBF calculation.

rCBF calculation

DSC MRI was not accurate for the measurement of absolute CBF values because of the inaccurate estimation of the contrast concentration in the vicinity of vessels and errors in the estimation of the microvascular hematocrit. Mukherjee et al. (52) demonstrated that better accuracy was achieved with rCBF by scaling to the white matter CBF. Therefore, rCBF has been used throughout this article to avoid systematic errors in the quantification of absolute flow rates. Assuming that the microvascular hematocrit is constant across the brain (53), rCBF was scaled by the CBF of white matter, which is known to have a fairly constant, age-independent flow of 20–22 mL/100 g/min (36).

Comparison of rCBF calculated using automated and manually selected AIF

Three regions of interest (ROIs) were randomly chosen and manually drawn on the cortical regions on each subject. The same ROIs were used for rCBF maps calculated from automated and manually selected AIFs. Care was taken to ensure that similar regions were drawn across subjects. Linear regression and Bland–Altman analysis of the mean ROI values from the two approaches were performed.

RESULTS

The general linear model was employed to perform a voxel-by-voxel univariate statistical test in the form of group comparisons (two-sample t-test) between patients with TBI and normal control subjects using AFNI’s 3dttest++ function. A false discovery rate threshold of 0.01 was selected in this study. As an additional effort to guard against false-positive results, only clusters that were greater than 500 voxels (500 mm$^3$) in volume are presented in the Results section. Statistical images representing significant group differences in rCBF are displayed as color overlays superimposed on the population-based $T_1$ template. Group differences in rCBF superimposed on the mean rCBF maps are presented in Appendix. The mean rCBF values of the clusters showing group differences were extracted and comparison of the mean rCBF values of these clusters was performed using an unpaired Student’s t-test.

Bivariate association of rCBF and neuropsychological data was evaluated by linear regression analysis using AFNI’s 3dRegAna function. Correlations were considered to be significant for $p < 0.01$. The mean rCBF values of the clusters demonstrating significant correlations with the neuropsychological data were extracted, and linear correlation analysis of the mean rCBF values of these clusters and the neuropsychological data was also performed. Only correlations with correlation coefficients larger than 0.60 ($R > 0.60$) are presented.

Figure 2 illustrates representative AIFs from a patient and a control subject. The automated and manual approaches generated very similar AIF curves. Figure 3 shows the rCBF maps of the same control subject (Fig. 3a, b) and the patient with mild TBI (Fig. 3c, d) calculated using the manually (Fig. 3a, c) and automatically (Fig. 3b, d) selected AIFs shown in Figure 2. It should be noted that the calculated rCBF maps from the automated AIF display the same features as the map obtained using the manually selected AIF for both the patient and control subject. The mean values of rCBF from the ROIs manually placed on the cortical regions for each subject demonstrated a strong linear correlation between the two approaches, with a regression coefficient of $R = 0.88$ and a slope of 0.88 (Figure 4a). The Bland–Altman plot (Fig. 4b) of the mean rCBF values also illustrated the good agreement between the two approaches. The mean difference between the two methods was $0.6 \pm 12.4$ mL/100 g/min.

Figure 2. Representative arterial input functions (AIFs) from a patient with mild traumatic brain injury (TBI) (crosses) and a control subject (full line). The automated (A, red) and manual (M, black) approaches generated very similar AIFs for the control and patient.
Voxelwise analysis of rCBF maps from each approach demonstrated multiple clusters of decreased perfusion \((p < 0.01)\) in the patient group compared with the normal controls. Table 3 summarizes the mean rCBF of the top five largest clusters with a minimum size of 500 voxels \((500\, \text{mm}^3)\) in volume, demonstrating reduced perfusion in patients with mild TBI by voxelwise group analysis. Figures 5 and 6 illustrate the group analysis results for subjects with mild TBI, showing decreased perfusion in the left cerebellum (Fig. 5, cluster 1), the left cuneus (Fig. 6, cluster 2), the right anterior cingulate (Fig. 6, cluster 3) and the right middle cingulate (Fig. 6, cluster 4) using both manually selected (top row) and automatically selected (bottom row) AIFs. The cluster numbers 1–4 listed in Figures 5 and 6 correspond to the numbered clusters listed in Table 3. The rCBF values from these four clusters were not statistically significantly different between blast and nonblast groups, as shown in Figure 7. (The patient groups with blast and nonblast indicate different conditions or categories.)

**Figure 3.** Relative cerebral blood flow (rCBF) maps using manually selected arterial input functions (AIFs) (a, c) agree well with rCBF maps calculated using automated AIFs (b, d) from both the control (a, b) and patient with mild TBI (c, d).

**Figure 4.** (a) Mean values of relative cerebral blood flow (rCBF) from selected regions of interest (ROIs) from each subject demonstrated a strong linear correlation between the two approaches with a regression coefficient of \(R = 0.88\). (b) The Bland–Altman plot also illustrated the good agreement between the two approaches. The mean difference between the two methods was \(0.6 \pm 12.4\, \text{mL}/100\, \text{g/min}\).
nonblast TBI did not demonstrate significant differences in their neuropsychological tests.) It is clear that the automated and manual approaches generated equivalent results for the evaluation of the perfusion deficits in patients with mild TBI. Therefore, only results from the automated approach are presented hereafter.

Voxelwise correlation analysis of rCBF demonstrated that MRI perfusion deficits in the cerebellum and anterior cingulate also correlated with the neurocognitive testing and neurobehavioral symptoms. Among all the neuropsychological test scores, the ANAM learning mean reaction time ($R = 0.61$), ANAM delayed memory mean reaction time ($R = 0.70$), CPT commission error T-score ($R = -0.70$), CPT detection T-score ($R = -0.66$), PCL-C total score ($R = -0.70$) and PCL-C avoidance score ($R = -0.68$). The anterior cingulate demonstrated significant correlations ($p < 0.01$) with the ANAM learning mean reaction time ($R = 0.71$), ANAM delayed memory mean reaction time ($R = 0.68$), PCL-C total score ($R = -0.76$), PCL-C re-experiencing score ($R = -0.71$), PCL-C avoidance score ($R = -0.70$) and PCL-C arousal/autonomic activation score ($R = -0.73$). The PAI depression score and PCL-C measurements are listed in Table 5. The rCBF values in the cerebellum and cingulate clusters were significantly lower in the patient group with PCL-C total scores higher than 44, as shown in Figure 9. The patient group with low PCL-C scores also demonstrated lower rCBF values at these clusters relative to normal controls (Fig. 9). In addition, the PAI depression scores also demonstrated a weak correlation with the perfusion values in the cerebellum ($R = 0.34$, $p = 0.05$) and anterior cingulate ($R = 0.44$, $p = 0.05$).

<table>
<thead>
<tr>
<th>Region</th>
<th>Size (mm$^3$)</th>
<th>Anatomical label</th>
<th>Patient rCBF (mL/100 g/min)</th>
<th>Control rCBF (mL/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>951</td>
<td>Left cerebellum</td>
<td>46.0 ± 8.5</td>
<td>79.1 ± 27.0</td>
</tr>
<tr>
<td>2</td>
<td>752</td>
<td>Left cuneus</td>
<td>39.7 ± 9.0</td>
<td>58.0 ± 11.1</td>
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<tr>
<td>3</td>
<td>713</td>
<td>Right anterior cingulate</td>
<td>32.9 ± 7.0</td>
<td>53.2 ± 18.2</td>
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<tr>
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<td>Right middle cingulate</td>
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<td>5</td>
<td>519</td>
<td>Left middle temporal gyrus</td>
<td>35.6 ± 10.0</td>
<td>54.3 ± 17.0</td>
</tr>
</tbody>
</table>

Figure 5. A cluster demonstrating decreased perfusion ($p < 0.01$) in the cerebellum in the patient group from the voxelwise analysis of relative cerebral blood flow (rCBF) maps in coronal (a, d), axial (b, e) and sagittal (c, f) views using manually (a–c) and automatically (d–f) selected arterial input functions (AIFs). Cluster 1 corresponds to the numbered cluster in Table 3.
DISCUSSION

Perfusion deficits in patients with mild TBI from a military population were characterized by DSC perfusion imaging using both manually and automatically selected AIFs. The AIFs selected from the automated approach demonstrated equivalent results to the corresponding AIFs selected by a trained operator. Both the linear regression analysis and Bland–Altman plot showed very good agreement between the mean values of the rCBF from the two approaches in selected ROIs. Both approaches detected multiple clusters of decreased perfusion in the cerebellum, cingulate, cuneus and temporal gyrus in the patient group with mild TBI in comparison with normal controls. This study demonstrates that rCBF can be confidently generated from the easier to use and more robust automated AIF approach. Although the rCBF maps calculated with the manual approach are not considered as a gold standard, this conventional approach is widely used and represents a reasonable evaluation of the accuracy of the automated method. Because automated AIF selection requires no operator input and allows rapid, standardized calculation of rCBF without the need for off-line image processing and specially trained personnel, it is anticipated that it will benefit clinical perfusion evaluations using DSC imaging.

Our group analysis results revealed scattered nonuniform regions of hypoperfusion in patients with mild TBI. The reduction in rCBF in the cingulate, cuneus and temporal gyrus, detected in the current study, is consistent with previous reports in patients with TBI (18,19,54,55). Voxelwise analysis also demonstrated significant rCBF reduction in the cerebellum in the patient group. To our knowledge, perfusion reduction in the cerebellum has not been reported previously in patients with TBI. One possible reason is that brain coverage of perfusion imaging is typically

Figure 6. Clusters demonstrating decreased perfusion ($p < 0.01$) in the cuneus and cingulate in the patient group from the voxelwise analysis of relative cerebral blood flow (rCBF) maps in coronal (a, d), axial (b, e) and sagittal (c, f) views using manually (a–c) and automatically (d–f) selected arterial input functions (AIFs). Cluster numbers 2–4 correspond to the numbered clusters in Table 3.

Figure 7. Patients with blast injuries demonstrated similar relative cerebral blood flow (rCBF) to patients with nonblast-related injuries at the four clusters showing decreased perfusion. Regions 1–4 correspond to the numbered clusters in Table 3.
limited because of the short time available for signal acquisition. Another important factor is that ROI analysis, rather than voxel-wise whole-brain analysis, uses predefined ROIs; the cerebellum is typically excluded from the predefined ROIs as it has classically been considered to be exclusively a motor control area. No frontal clusters demonstrating significantly reduced perfusion survived the cluster size correction, which was possibly caused by the severe susceptibility artifacts in this region.

Neuropsychological deficits, especially slowed reaction time, are reported at the acute stage of mild TBI. Slower reaction times during reaction time tasks have been reported to correlate with abnormal MR findings by Hofman et al. (56) in patients with mild TBI. Neuropsychological deficits are not typical beyond the very acute period. Nevertheless, Ge et al. (57) demonstrated reduced thalamic perfusion correlated with several neurocognitive measures, including processing and response speed, and memory/learning, in patients 24.6 months after injury. In our study, the right anterior cingulate gyrus and the cerebellum region showing decreased perfusion demonstrated positive correlations with the mean reaction time in ANAM learning. The anterior cingulate cortex has been implicated in cognitive processes, including divided attention, novelty detection, working memory, memory

Table 4. Correlations of perfusion measures with neurocognitive data and neurobehavioral symptoms

<table>
<thead>
<tr>
<th>Region</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left cerebellum</td>
<td>Right anterior cingulate</td>
<td></td>
</tr>
<tr>
<td>ANAM learning</td>
<td>$R = 0.61$</td>
<td>$R = 0.71$</td>
<td></td>
</tr>
<tr>
<td>ANAM delayed memory</td>
<td>$R = 0.70$</td>
<td>$R = 0.68$</td>
<td></td>
</tr>
<tr>
<td>CPT commission error</td>
<td>$R = -0.70$</td>
<td>$R = -0.66$</td>
<td></td>
</tr>
<tr>
<td>CPT detection</td>
<td>$R = -0.66$</td>
<td>$R = -0.66$</td>
<td></td>
</tr>
<tr>
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<td>$R = -0.70$</td>
<td>$R = -0.76$</td>
</tr>
<tr>
<td></td>
<td>$R = -0.68$</td>
<td></td>
<td>$R = -0.71$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$R = -0.73$</td>
</tr>
</tbody>
</table>

ANAM, Automated Neuropsychological Assessment Metrics; CPT, Continuous Performance Test; PCL-C, Post-traumatic Stress Disorder Checklist-Civilian Version.

Table 5. Post-traumatic Stress Disorder Checklist-Civilian Version (PCL-C) measurements and Personality Assessment Inventory (PAI) depression score

<table>
<thead>
<tr>
<th>Neuropsychological measurement</th>
<th>Test score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL-C total score</td>
<td>$38 \pm 14$</td>
</tr>
<tr>
<td>PCL-C re-experiencing</td>
<td>$11 \pm 5$</td>
</tr>
<tr>
<td>PCL-C avoidance</td>
<td>$15 \pm 7$</td>
</tr>
<tr>
<td>PCL-C arousal/autonomic activation</td>
<td>$12 \pm 4$</td>
</tr>
<tr>
<td>PAI depression score</td>
<td>$61 \pm 14$</td>
</tr>
</tbody>
</table>
retrieval, evaluative judgment, motivation and performance monitoring (58–62). From the large number of neuropsychological tests, a larger number of commission errors on CPT was the only measure which differed significantly between the controls and patients with mild TBI 108 months after injury (63). Our results demonstrated that the larger number of commission errors was correlated with decreased perfusion in the cerebellum region. Recently, there has been increasing evidence that the range of tasks associated with cerebellar activation is significant, including tasks designed to assess attention, executive control, language, working memory, learning, pain, emotion and addiction (64). Our previous work evaluating the working memory of patients with TBI in the same military population also demonstrated altered activation in the cerebellar region (65). These preliminary results suggest that the cingulate and cerebellum could be regions that warrant more attention in the evaluation of patients with TBI in future studies.

Our results also demonstrated significant negative correlations between rCBF in the left cerebellum and right anterior cingulate and the neurobehavioral symptoms of PTSD. The rCBF values at the cerebellum and cingulate clusters were significantly lower in the patient group with PCL-C total scores higher than 44. PTSD and traumatic brain injury often coexist (66), because brain injuries are often sustained in an emotionally traumatic context. Recent evidence has suggested that some of the impairment secondary to mild TBI can be largely attributable to stress reaction after TBI (67,68). In addition, the PAI depression scores also demonstrated a weak correlation with the perfusion values in the cerebellum and anterior cingulate, suggesting that depression might also have an impact on cerebral perfusion in patients with TBI. These results suggest that the presence of PTSD and depression in the context of mild TBI is associated with greater deficits than mild TBI alone. However, the presence of PTSD would not undermine the hypothesis that perfusion deficits are related to functional changes in mild TBI. Given the complexities of co-morbidities in these patients, it is unclear of the role played by mild TBI in the etiology or expression of these clinical symptoms. Even in those subjects that fell below the levels consistent with diagnosable depression or PTSD, significant differences were found relative to control subjects. Some authors have found differences between blast and nonblast TBI in terms of symptom expression (69,70). Several studies have found no difference in neuropsychological profiles, however (71,72). The rCBF values from the left cerebellum, left cuneus, right anterior cingulate and right middle cingulate were not statistically significantly different between blast and nonblast groups in this study.

Caffeine abstinence was not required for this study as previous research has suggested that it is best that patients consume their regular daily amount of caffeine before imaging when studying cerebral perfusion. Because the brain adapts to the level of caffeine typically consumed and adjusts CBF accordingly, simply telling people not to drink caffeinated beverages before the scan could further skew the results (73). Smoking was also not controlled in the study because a number of reports have demonstrated that there is no significant difference in rCBF between young smokers and nonsmokers (74,75). However, there are potential confounders that might affect the results. Various medications were prescribed to the patients with TBI at the time of MRI. Among the commonly prescribed medications in Table 2, laxatives, antibiotics and antiemetics have no known effect on cerebral perfusion. However, some pain relievers and anticonvulsants can potentially change cerebral perfusion. Nevertheless, the alterations are not consistent across the brain region and different medications can have opposite effects (76,77). Therefore, it is very difficult to isolate the effects of these medications. As these are common medications for military patients with TBI, the results of this study are likely to be applicable to other military TBI populations. A global normalization procedure has been used in the current study. Recent research suggests that a decrease in cortical CBF in conjunction with normalization to the global mean can lead to spurious findings of elevated subcortical rCBF (78,79). Even though global hypoperfusion has been detected in patients with chronic TBI (54), most of the patients participating in this study were in the subacute stage rather than the chronic stage.

Our study has several limitations. First, the patients seen at WRAMC may not be typical of the typically deployed service member with TBI. Individuals are evacuated to WRAMC because of significant bodily injury or other complicating factors. Second, the neuropsychological tests were performed in a 2-week window of MRI, which may lead to potential bias of correlation. Third, clinical and administrative factors prevented us from using a control group with a history of deployment or extracranial injuries without TBI, making it harder to separate other possible deployment-related health conditions from mild TBI. However, our findings suggest that, although stress plays an important role in the phenotype of these individuals, it does not explain...
the full clinical picture. Likewise, the subjects enrolled in this study reflect the clinical reality of the patients seen at WRAMC. Although ‘pure’ mild TBI might better illustrate our findings, it would not reflect the typical military patient with TBI and would limit the generalizability of our findings.

In conclusion, rCBF calculated using AIFs selected from an automated approach demonstrated good agreement with the results using manually selected AIFs. Preliminary group analysis of patients with mild TBI from a military population demonstrated scattered perfusion deficits using DSC imaging. Furthermore, perfusion deficits in the cerebellum and anterior cingulate showed significant correlations with measures of verbal memory, speed of reaction time and self-report of stress symptoms.

Acknowledgements

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REFERENCES


APPENDIX

Figure A1. A cluster demonstrating decreased perfusion ($p < 0.01$) in the cerebellum in the patient group from the voxelwise analysis of relative cerebral blood flow (rCBF) maps overlaid on the mean rCBF images in coronal (a, d), axial (b, e) and sagittal (c, f) views using manually (a–c) and automatically (d–f) selected arterial input functions (AIFs). Cluster 1 corresponds to the numbered cluster in Table 3.

Figure A2. Clusters demonstrating decreased perfusion ($p < 0.01$) in the cuneus and cingulate in the patient group from the voxelwise analysis of relative cerebral blood flow (rCBF) maps overlaid on the mean rCBF images in coronal (a, d), axial (b, e) and sagittal (c, f) views using manually (a–c) and automatically (d–f) selected arterial input functions (AIFs). Cluster numbers 2–4 correspond to the numbered clusters in Table 3.