Automated Detection and Quantification of Brain Lesions in Acute Traumatic Brain Injury Using MRI

F. G. Hillary · B. B. Biswal

Abstract The purpose of this study is to develop a reproducible method for quantifying brain lesions in traumatic brain injury (TBI). Quantifying the effects of neuropathology is an important goal in the study of brain injury and disease, yet examiners have encountered significant difficulty quantifying brain lesions in neurotrauma where there may exist multiple, overlapping forms of injury including large focal lesions and more subtle, diffuse hemorrhage and/or shear injury. In the current study, we used conventional MRI to quantify brain lesion volume at separate time points in individuals with severe TBI. We present an automated method (ISODATA) for quantifying brain lesions that is compared against a standard semi-automated volumetric approach. The ISODATA method makes no assumptions about the location or extent of brain lesions, instead identifying areas of neuropathology via voxelwise comparisons of MRI signal intensity. The data reveal that ISODATA overlaps significantly with a semi-automated approach, is reliable across multiple observations, and is sensitive to change in lesion size during recovery from TBI. This study validates a reproducible, automated lesion quantification method used here to determine the location and extent of brain pathology following TBI. This approach may be used in conjunction with advanced imaging techniques to characterize the relationship between brain lesions and neurometabolism and function.

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Introduction

Detection of brain injury and disease using MRI techniques has improved dramatically over the past decade. At least part of this advancement is attributable to novel methods providing the opportunity to discriminate between small differences in MRI signal intensity on a voxelwise basis. Quantifying brain pathology remains an important clinical and research goal in understanding the effects of brain injury and disease, yet rapid, objective quantification of brain lesions remains elusive. Semi-automated procedures requiring visual inspection and manual tracing of ostensible lesion sites have been used to examine the effects of a number of pathophysiological processes including HIV (Itti 2001), multiple sclerosis (MS) (Molyneux et al. 2000; Raff et al. 2000; Rovaris et al. 1999), and tumor (Xie et al. 2005). Manual procedures, however, are typically time consuming and often require assumptions about the extent and consistency of the tissue for any brain area identified as a "lesion".

In the study of neurotrauma, semi-automated volumetric procedures have been used extensively in mild traumatic brain injury (TBI) documenting changes in ventricular size and in sulcal/gyral relationships months to years post injury (for a review of these quantitative MRI methods in mTBI see Bigler (2001)). Many of these studies employed semi-automated approaches requiring manual identification of anatomical parameters and, because of the mild nature of the injuries, did not focus directly on lesion quantification. In contrast, identifiable brain lesions are present in moderate to severe TBI (nearly by definition) and the

pathophysiology results in varying forms of injury (e.g., contusion, axonal shear injury, hemorrhage) that may occur in isolation or coexist depending upon the mechanism of injury and injury severity (Whyte et al. 1998). Because neurotrauma is often diffuse and inconsistent across a defined "lesion space", lesion identification via visual inspection is difficult and occasionally unreliable.

Automated MRI procedures

Advances in MRI data analysis now provide the opportunity to quantify brain lesions secondary to injury and disease by using automated segmentation or tissue classification algorithms. Fully automated algorithms have been developed to segment gray and white matter and have been used to quantify demyelinating and ischemic processes (Hasselblatt et al. 2003; Van Leemput et al. 2001; Zijdenbos et al. 1994; Zijdenbos et al. 2002). More recently, investigators developed automated methods for segmenting gray matter areas to isolate and quantify cortical dysplastic lesions (Srivastava et al. 2005). This method was useful for detecting larger lesions (53%, median of 7.2 cm^3), but was increasingly unreliable for smaller lesions and failed to detect most lesions under 3 cm^3. Much of the work to date using these types of automated methods has focused on quantifying discrete lesions that maintain a relatively clear demarcation from adjacent healthy neural tissue. Because of this, it is unclear if similar algorithms would be successful in TBI where lesions may be both focal and diffuse, varying in both size and signal intensity.

Important work by Anbeek and colleagues resulted in an automated brain lesion quantification method in individuals with MS by integrating information from five separate MRI sequences (Anbeek et al. 2004) (T1 weighted, inversion recovery, proton density, T2-weighted, and fluid attenuation inversion recovery). The results of this automated method showed significant overlap with a traditional semi-automated procedure revealing good estimation of the location and extent of white matter degradation without any information *a priori* about potential lesion sites.

The algorithm used by Anbeek and colleagues was a K-nearest neighbor classification technique for clustering; spatial information about intensity of clusters of voxels were integrated across the separate images, with each image contributing distinct information about each voxel based upon its MRI weighting (i.e., contrasts). This method required no information about potential lesion locations, increased reliability compared to a semi-automated approach, and was generally sensitive to lesions of varying sizes. One shortcoming to this method is that because it integrates data from five separate MRI sequences for lesion quantification, minimization of head motion between each

MRI scanning sequence is essential. In clinical samples, head motion is typically greater compared to healthy adults (Krings et al. 2001) and efforts to minimize head movement are even further challenged in cases of acute TBI. Head stabilization of minimally responsive patients is time consuming and, depending upon the stage of coma recovery, recognizable head movement is not uncommon. For this reason, lesion detection and quantification in TBI would ideally occur with rapid acquisition using a single conventional MRI sequence.

Quantifying brain lesions in TBI in the current study

We present a reproducible method for detecting and quantifying trauma-induced brain lesions. In doing so, there are three important goals in this study: 1.) to develop an automated method that detects and quantifies lesions using a single conventional clinical MRI scan, 2.) to validate the proposed automated method (Iterative Self-Organizing Analysis Technique, ISODATA) by comparing it to the current "gold standard" for lesion detection method requiring manual lesion identification, and 3.) to use ISODATA to measure changes in brain lesion size across separate time points during recovery from severe TBI. The purpose of this last aim is to assess the sensitivity in the proposed method in detecting changes in brain lesion size during the acute TBI recovery period when gross lesion resolution is expected and observable using conventional structural MRI. To our knowledge, this is the first study to use automated methods for identification and quantification of brain lesions secondary to brain trauma and first of any kind to quantify acute neurotrauma. The method proposed here allows for quantification of neurotrauma and lesion resolution over time, ultimately affording examiners the opportunity to study the relationship between observable brain lesions and indices of brain structure, metabolism, and function.

Materials & methods

Participants and inclusion/exclusion criteria

Study participants consisted of five healthy adults and five males between the ages of 19 and 55 sustaining severe TBI. In regards to the TBI sample, two subjects were in a vehicle during a motor vehicle collision, two subjects were bicyclists struck by a motor vehicle and the most severe case (#2) was the victim of an assault. Participants in the group of individuals with TBI had a definitive diagnosis of TBI, defined by the CDC as (Harrison-Felix et al. 1996): "damage to brain tissue caused by an external mechanical force, as evidenced by loss of consciousness due to brain

trauma, post-traumatic amnesia, skull fracture, or objective neurological findings that can be reasonably attributed to TBI on physical examination or mental status examination". TBI severity was defined by the best Glasgow Coma Scale (GCS) score (Teasdale and Jennett 1974) during the first 24 h after injury and scores ranging from 3–8 were considered "severe". For this sample, all subjects had initial GCS scores of 7 or less and all subjects but one maintained essentially stable GCS scores during the first 72 h following injury. Four subjects remained non-responsive and one was responsive, with significant posttraumatic confusion at the time of the first MRI scan. CT/MRI findings were positive for all subjects and these were the sites for lesion quantification.

Inclusionary/exclusionary criteria

Individuals were excluded from this study if they had been previously informed that it was unsafe to receive MRI for clinical treatment. Participants with potentially ferrous material in their bodies including but not limited to metal clips to repair a cerebral aneurysm, pace-makers, Baclofen pumps, and cochlear implants were excluded. The criteria for MRI investigation developed by the Department of Radiology for everyday clinical use was followed in this study in order to determine such exclusions.

Patients were required to be medically stable with Systolic Blood Pressure >=90 mmHg at the time of MRI scanning. Patients were excluded from the study if they had life threatening dysrythmias, chronic pre-existing renal or hepatic impairment, known spinal cord injury, persistent hemodynamic instability, or penetrating head injury. In addition, patients were excluded from the study if they had a history of previous neurologic disorder such as head injury or seizure disorder, or significant neurodevelopmental psychiatric history (such as schizophrenia or bipolar disorder). All of these exclusions were covered in the IRB approved consent form and were discussed with the family members of each study participant.

General procedure

The goal of this study was to use ISODATA in conjunction with AFNI software (http://afni.nimh.nih.gov/afni/) to identify and quantify brain lesions at two separate time points. To do so, the following steps were followed: 1.) family interview to determine if the patient was a potential candidate for the study and with IRB approved consent, review of medical records, 2.) two MRI scans, with scan one occurring during the first week of recovery and the second scan occurring approximately 3 weeks post injury. Once the patient was medically stable, initial contact was made with family members and an interview was con-

ducted. With informed consent, medical records were reviewed to record clinical information regarding the nature and magnitude of the brain injury. If the patient was deemed appropriate for this study, the first MRI scanning session was performed and the second scan within 2 weeks of the initial MRI scan.

Neuroimaging procedures

All MRI data acquisition was carried out on a 1.5 GE MRI Signa scanner (General Electric Medical System, Milwaukee, WI) using available product software. Imaging sequences included T1-weighted sagittal and coronal localizers (TE= 16 ms, TR=500 ms, 256×128 matrix, 5 mm thick slices with a 2.5 mm gap) and a conventional FLAIR (TE, echo time= 140, TR, repetition time=1,000, TI, time of inversion=220, FOV=20 cm, 5 mm/skip 0) composed of 24 slices providing whole brain coverage. In order to avoid altering lesion data, there was no registration performed for comparing between Time 1 and Time 2 MRI scans. To permit comparison between time points, for all subjects, the 11th slice was aligned obliquely with AC-PC.

All subjects were taken to the MRI suite at the same time of day (approximately 7 am) and prepared for scanning with the assistance of the trauma surgery and respiratory therapy staff. Study participants were transferred to the MRI gurney and their heads were stabilized with foam cushioning. All participants were in stable medical condition and 4/5 were non-responsive at the time of the first scan. The total time from departure to return to the surgical intensive care unit was 1 1/2 to 2 h.

Data analysis

A multiparametric method, ISODATA, was used to identify brain lesions in the subjects with TBI. As noted, the ISODATA technique is an unsupervised classification method that allows for categorization of an open-ended number of clusters, or tissue classes. The ability to adjust the number of potential dissociable clusters is the primary advantage of the ISODATA method because it requires no knowledge *a priori* of the exact number of clusters or their locations prior to segmentation. Similar multiparametric methods have been used in stroke studies (both in animal models and human subjects) to demonstrate evolving heterogeneity within lesions (Jacobs et al. 2001a, b; Mitsias et al. 2002).

The ISODATA method proposed here allows for unsupervised classification of multi-dimensional data sets (e.g., 2-D, 3-D) and it is an iterative procedure, where the maximum number of clusters can be predetermined. Each voxel in the image is then compared to the average value of the cluster to which it belongs. A mean cluster value is

determined using voxels from each of the clusters and the number of potential clusters is then determined by the algorithm. This procedure is repeated for all data points until the Mean Squared Error (MSE) is minimized in each of the clusters (see Equation 1 below). The ISODATA algorithm thus allows for measurement of subtle, within-subject differences in voxel intensity, which can then be used to identify the lesions in an unbiased fashion. Moreover, the ISODATA method used here does not differentiate clusters through the use of an absolute threshold, which increases its flexibility and permits detection of subtle distinctions in tissue classes. ISODATA thus uses a soft threshold for clustering and a weighting strategy that incorporates information about the immediate environment of any cluster (e.g., cluster surrounded by similar or dissimilar tissue).

The equation for calculating Mean Squared Error in each of the clusters is:

$$MSE = \Sigma[x - c(x)]^2 \Big/ N - C \qquad (1)$$

where x = a single voxel, c(x) is the mean value of cluster c, N is the number of voxels in the slice, and C is the number of clusters

In the current study, parameterization of lesion areas was achieved by analyzing, on a slice-by-slice basis, the brain lesions evident across the five cases of severe brain trauma. Each MRI slice containing a brain lesion determined via ISODATA analysis was considered an individual observation (or distinct lesion or set of lesions), resulting in 38 total observations across the five subjects. During analysis ISODATA was permitted to extract up to ten different tissue classes for any given slice. Because of this, there were no geometric constraints for ISODATA and no minimum voxel threshold was imposed for any given cluster. That is, ISODATA was allowed to find ten tissue classes in every slice, so no minimum to the number of voxels per cluster was needed.

Comparing semi-automated and ISODATA procedures

In order to establish that ISODATA accurately categorizes brain lesions as a separate class of tissue, findings were compared with a "gold-standard" (GS) derived from the results of two experts using a semi-automated procedure. In the semi-automated procedure, lesions were manually traced by a neurosurgeon and a radiologist, both with clinical experience in the recognition and diagnosis of neurotrauma. This manual procedure required lesion identification for all slices in the five subjects. Examiners conducted manual tracing of lesioned areas using AFNI software given the following instruction: "Identify all areas

of lesion. The areas identified should reflect pathology/injury including areas of contusion, edema, and/or hemorrhage". The experts were not aware of the findings produced by ISODATA, performed their ratings independent of one another, and were permitted to examine all brain slices in order to determine those where lesions were evident and to create lesion "masks" via manual tracing. Total lesion volume was quantified for both the semi-automated and automated procedures using AFNI software. There were a total of 38 slices with identifiable lesions for comparison.

It is important to note that in order to examine lesion overlap between methods, ISODATA was used for only those slices where lesions had been identified in the gold standard. The reason for this is that "healthy" slices containing no lesion do not provide the opportunity to examine voxel overlap between the methods (i.e., "overlap" when no lesion is found is zero). Of critical emphasis, however, is that ISODATA was required to examine voxels representative of healthy tissue; during these analyses ISO makes hundreds of thousands of decisions whereby non-lesion and lesion "tissue" are examined simultaneously.

Determining spatial overlap used a simple algorithm measuring the intersection between distinct areas. For example, the spatial overlap between expert raters, or the "gold-standard" (GS) was determined by examining the total number of voxels identified as lesion by both examiners (see Equation 2):

$$SA = (E1 \cap E2) \qquad (2)$$

Spatial overlap between the two expert raters, where: E1 = # of voxels determined by Expert 1, E2 = # of voxels identified by Expert 2, and $\cap$ = the intersection of these regions.

An identical method for determining the GS (i.e., overlap in results between two experts) was used to compare the GS with the ISODATA results (spatial overlap = ISO $\cap$ GS, where ISO is the ISODATA result and GS is the representative gold standard).

It should be noted that this algorithm does not account for additional voxels included by only one of the two "raters" (e.g., voxels included by ISODATA, not included by Expert rater 1). The reason for permitting the potential for "over-inclusion" of voxels was that it was a primary goal to locate all voxels that are statistically "abnormal". Because ISODATA can isolate individual lesion clusters, thus providing specificity, we were interested in guaranteeing high sensitivity at the outset (i.e., maximal overlap with semi-automated procedures at the expense of commission errors). For this reason, we used a simple procedure to determine overlap, with the primary analysis focused on determining if voxels represented in the GS were evident in the ISODATA mask.

Examining reliability of ISODATA

In order to determine the reliability of ISODATA in identifying tissue classes (i.e., clusters) for any given slice, the image quality of the original FLAIR image was altered by adding three levels of random noise (e.g., 5%, 15%, and 25%) averaged over 50 iterations. In order to directly manipulate image quality, white Gaussian noise with differing distributions, or variance, was added to each of the 38 slices. The ISODATA algorithm was then used to assign each voxel to a tissue cluster over 50 repetitions. That is, ISODATA assigned each voxel to a cluster in each slice and the mean and standard deviation of the clusters and their respective size was determined. The goal was to determine if this cluster assignment was altered due to image quality. Analyses were performed at each of level of added noise by using the original cluster assignment at each voxel (e.g., between 1 and 20) in the original image to predict the tissue cluster assignment of the identical voxel after adding noise. These simulations provided the opportunity to increase the number of observations and to examine the reliability of ISODATA across varying image quality.

Histogram analysis

Histogram analyses were used in order to display the differences in the signal intensity distributions between groups. Histogram results were compared between observations (slices) with and without lesions in order to visualize basic differences in the distributions between slices with and without brain lesion.

Results

The results are separated into sections related to: 1.) the cross-validation of ISODATA via comparison with expert ratings, 2.) examination of the reliability of ISODATA across three levels of noise simulation, and 3.) examination

of the changes in brain lesion size from the first to the second MRI scan using ISODATA.

Validation of ISODATA

Histogram analysis—lesion identification

As noted, we employed an open-ended procedure for determining the number of potential tissue classes; ISODATA was permitted to identify up to ten distinct tissue classes. This approach allowed us to examine the various clusters categorized by ISODATA as separate tissue types without restrictions regarding the number of potential solutions. Based upon voxel signal intensity, ISODATA consistently separated three tissue classes in healthy adults and six tissue classes in individuals with TBI. The three additional tissue classes in TBI correspond to distinct lesion subtypes (Table 1).

The histogram results in Fig. 1b, d represent the distribution of voxels for an axial slice in a healthy adult and a participant with TBI, respectively. The lowest MRI signal intensity represents signal from CSF in ventricles, areas between sulci, and areas around the perimeter of the brain parenchyma. In both groups, separation between CSF and gray/white matter, occurred at an MRI signal intensity of from 40–60 (again, see Fig. 1b, d). The highest MRI signal intensity, observed as the right tail in the distribution, is absent in healthy adults compared to individuals with TBI. Figures 2a and b offer histogram results demonstrating the range of voxel intensities in clusters representing CSF, gray/white, and lesion in two cases of TBI.

ISODATA and semi-automated approach

ISODATA revealed three separable lesion clusters and these results were compared separately and in aggregate with the *total* lesion volume determined using the GS (see Table 1). When considering total ISODATA lesion load, there was 90% overlap with the GS. Figure 3 shows a FLAIR image of a large left frontal contusion (4A), the associated

Table 1 Reveals the overlap between ISODATA results and the semi-automated approach

Slice	#	L1	L2	L3	Total	ER 1	ER 2	GS	ISO L1 vs GS[a]	ISO L1L2-vs GS[a]	ISO total vs GS[a]
1	11	38,250	24,380	20,916	83,546	73,287	77,928	68,764	44.9	69.3	94.6
2	4	746	428	249	1,423	1,294	1,572	1,086	44.6	79	87.1
3	13	5,671	3,679	2,474	11,824	9,665	7,616	6,846	63.8	89.2	91.9
4	2	2,813	1,896	871	5,580	2,900	3,151	403	47	77.1	85.3
5	8	5,875	3,844	1,191	10,910	8,647	10,297	7,507	67.5	82.2	94.1
								Avg:	53.6	79.4	90.6

= number of slices, or observations averaged, L1 = ISODATA lesion 1 (third highest voxel intensity), L2 = ISODATA lesion 2 (second highest voxel intensity), L3 = ISODATA lesion 3 (highest voxel intensity). [a] values represent percent overlap.
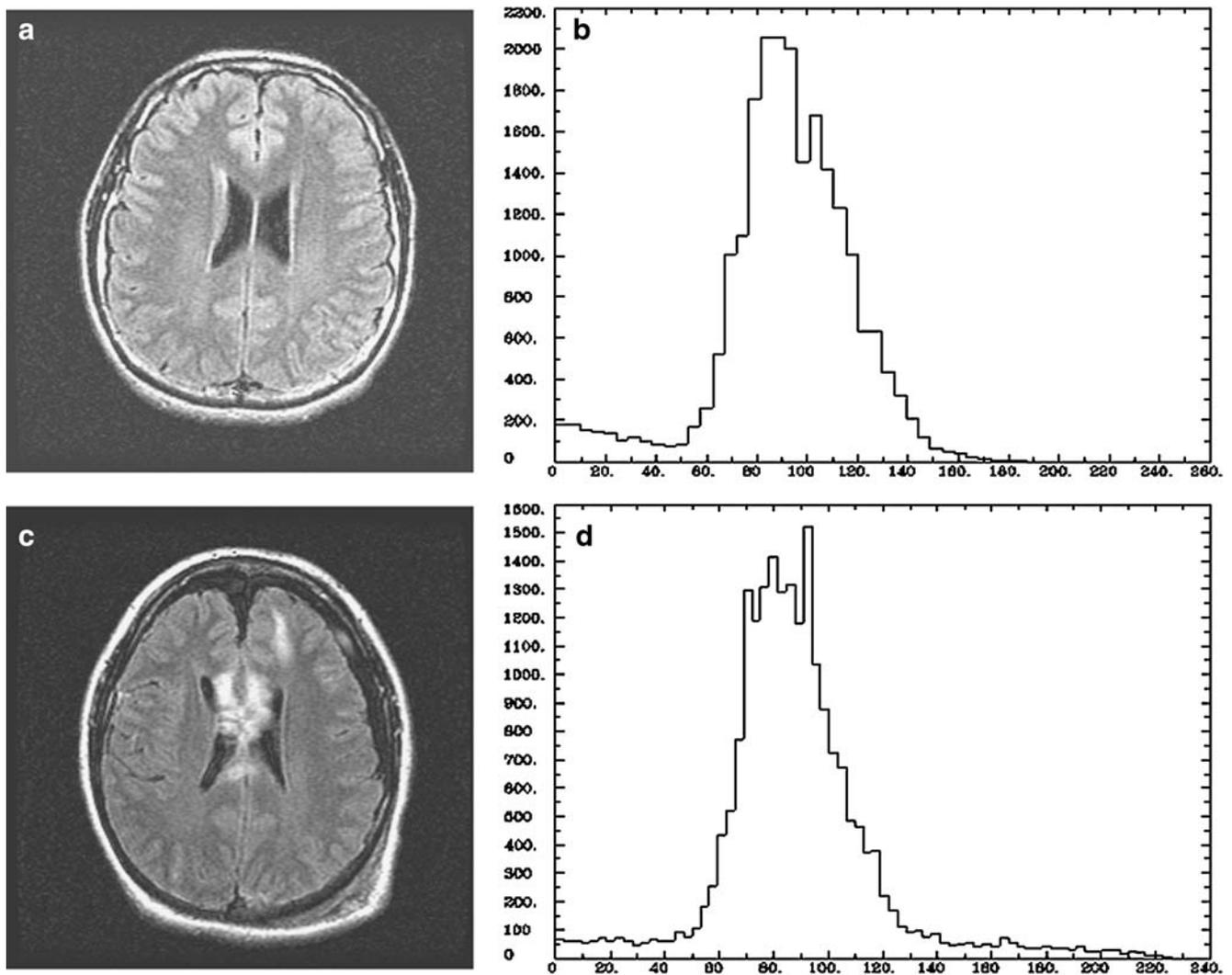
Fig. 1 Illustrates the differences in the voxel distributions between a healthy adult and an individual with TBI. In these images, **a** is a FLAIR of a healthy adult, **b** is the distribution of all voxels in the FLAIR shown in A, **c** is a FLAIR of an individual with TBI, and **d** is the distribution of all voxels in the FLAIR image shown in C. Note the extended "right tail" of the distribution in D; individuals with TBI were consistently differentiated from healthy adults by this group of voxels with very high MRI signal intensity

ISODATA results (4B), the results from the semi-automated approach (4C), and the areas of union and intersection (4E). The overlap observed here between ISODATA and the semi-automated GS is comparable to other studies examining automated and semi-automated approaches (Zijdenbos et al. 1994; Anbeek et al. 2004).

A second histogram series was used to overlay the four sets of results for the semi-automated approach (e.g., expert ratings, GS, ISODATA). Figure 4 shows the number and intensity of voxels identified as lesion for each of the methods for each of the five subjects. To visualize the results, the 38 slices were collapsed and presented as averages for each subject. In all cases, ISODATA (red) demonstrates greater

inclusion of "abnormal" voxels compared to the GS (green).

Examining reliability of ISODATA: noise simulation

The reliability of the ISODATA findings was assessed by examining the size and extent of areas determined to be lesions after adding one of three levels of random noise: 5%, 15%, and 25%. Random noise was added to each slice individually, permitting 38 comparisons between the original and simulated data. To do so, each voxel was assigned a cluster, or tissue class, between 1 and 20 by ISODATA and this tissue classification in the original

Fig. 2 a and **b** Two subjects with respective histogram results for all voxels in the axial slice shown. Partitioned areas within the histogram results correspond with values for CSF, gray/white, and lesion in the image to the right
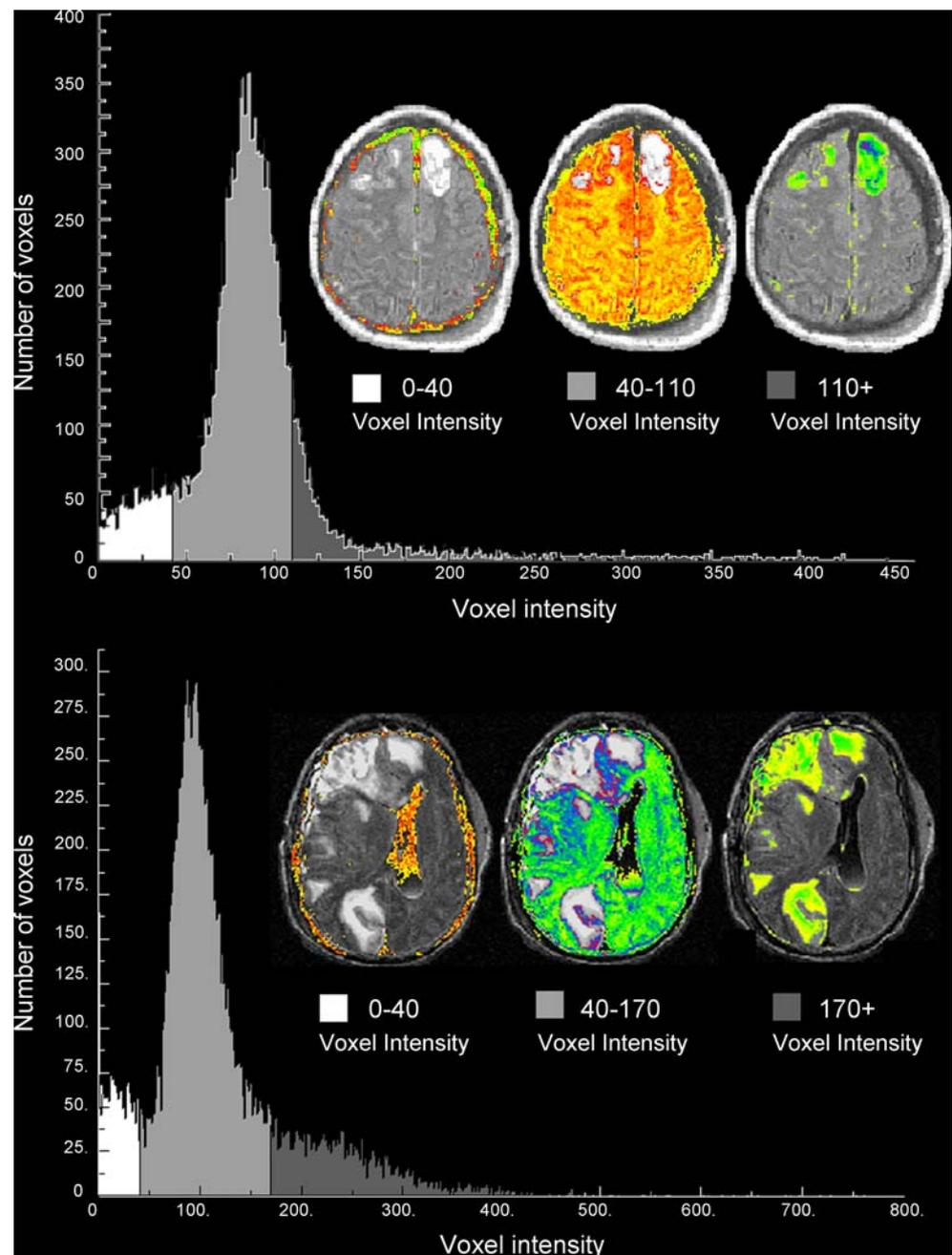
voxel was used to predict the tissue classification after the addition of noise. Table 2 shows the results comparing the original ISODATA findings with the three levels of simulated noise (averaged for each subject). The r^2 values in Table 2 indicate the relationship between the original tissue classification in the standard image with the tissue classification in an image with one of three levels of noise added. Using each voxel as its own regressor, the cluster assignment by ISODATA in the original data, consistently predicted >95% of the variance in the

simulated data. The findings here indicate that ISODATA can reliably categorize tissue classes even given differences in image quality.

Detection of changes in lesion size

To provide an example of a clinical research application for the use of ISODATA to examine lesion size and subtype, the current study acquired separate FLAIR images during a time period when gross lesion resolution was expected.

Fig. 3 Illustrates the typical overlap between the semi-automated and the automated (ISODATA) method. **a** depicts a left prefrontal lesion (radiologic convention) and **b** and **c** show the areas determine to be lesion by ISODATA and the gold-standard, respectively. Note that in **b**, ISODATA separates the lesioned area into three distinct lesion categories based upon signal intensity and in **c**, lesion is dichotomous. Finally, in **d**, areas of maroon indicate the intersection, or overlap, between methods and areas of green area contain the total number of voxels that were contained in ISODATA, but not the gold-standard

T1 FLAIR

ISODATA

Gold-standard

Union/Intersection
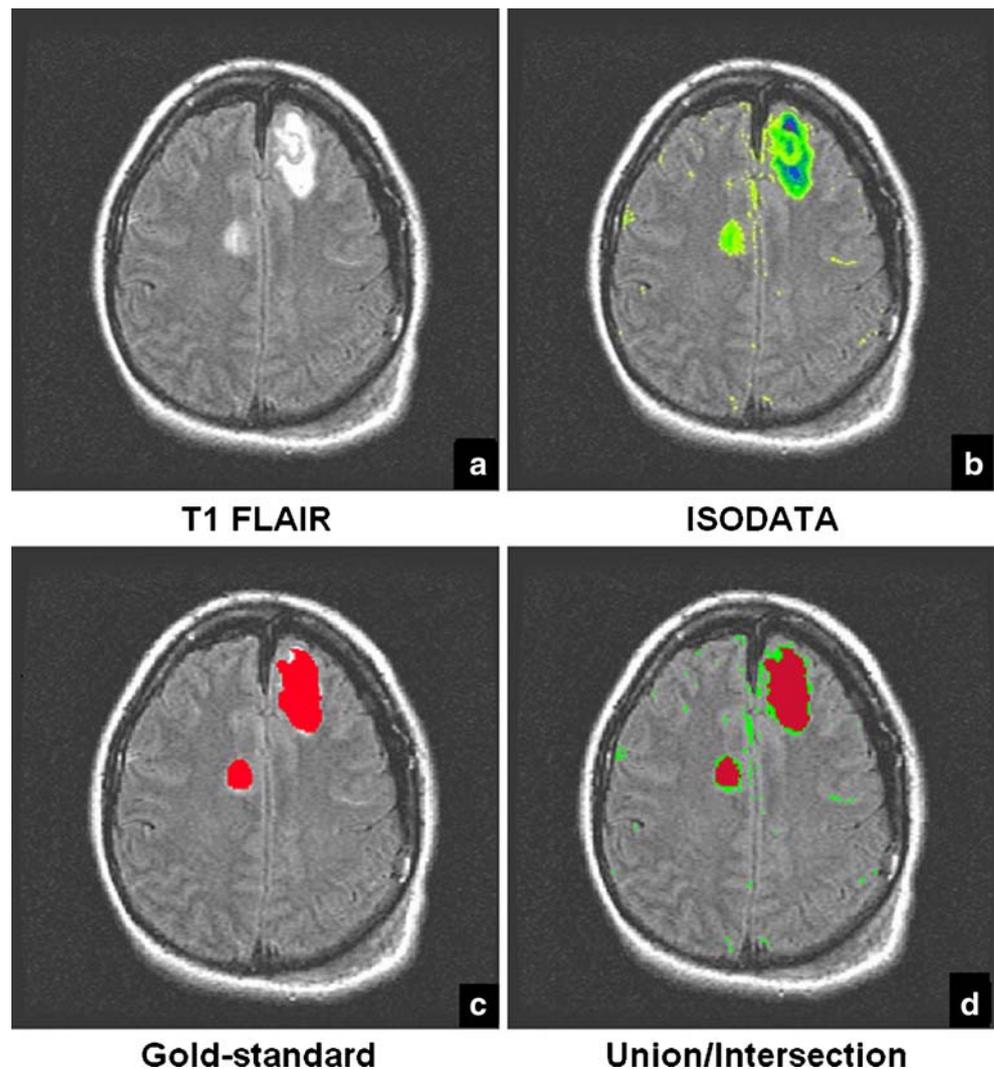
Sites identified as lesions by ISODATA decreased in size for all but one of the slices (see Fig. 5 for average change in each of the lesion subtypes); the average total lesion volume decreased by 61% (SD=0.237) from Time 1 to Time 2. Again, when considering the individual lesion classes identified by this method, ISODATA consistently identified three significant clusters at both time points.

Finally, Figs. 6 and 7 illustrates the changes in the size of the lesion and the histogram results at Time 1 and Time 2. These data demonstrate that the changing lesion size is reflected in the reduction in the number of hyperintense voxels (e.g., right tail of the distribution) and ISODATA lesion identification mirrors this lesion resolution.

Discussion

The primary goal of this study was to validate a reproducible method for automated detection and quantification of brain lesions in severe TBI. To our knowledge,

there has been no work to date using automated techniques to quantify lesions secondary to moderate and severe TBI during the acute recovery period. In the following, we first discuss the validation of ISODATA, followed by the potential advantages for using ISODATA to examine moderate and severe TBI.

Validation of ISODATA

Because an automated procedure like ISODATA has not been used to examine TBI, one goal was to compare this method to the current GS, a traditional semi-automated lesion quantification method. This comparison revealed good overlap (average of 0.90) and these data are consistent with examinations in MS and stroke comparing automated and semi-automated volumetric approaches (Zijdenbos et al. 1994; Anbeek et al. 2004; Krings et al. 2001; Jacobs et al. 2001b). The purpose of this comparison was to confirm that the hyperintense voxels detected by ISODATA were areas of brain lesion and not artifact, CSF, or healthy white
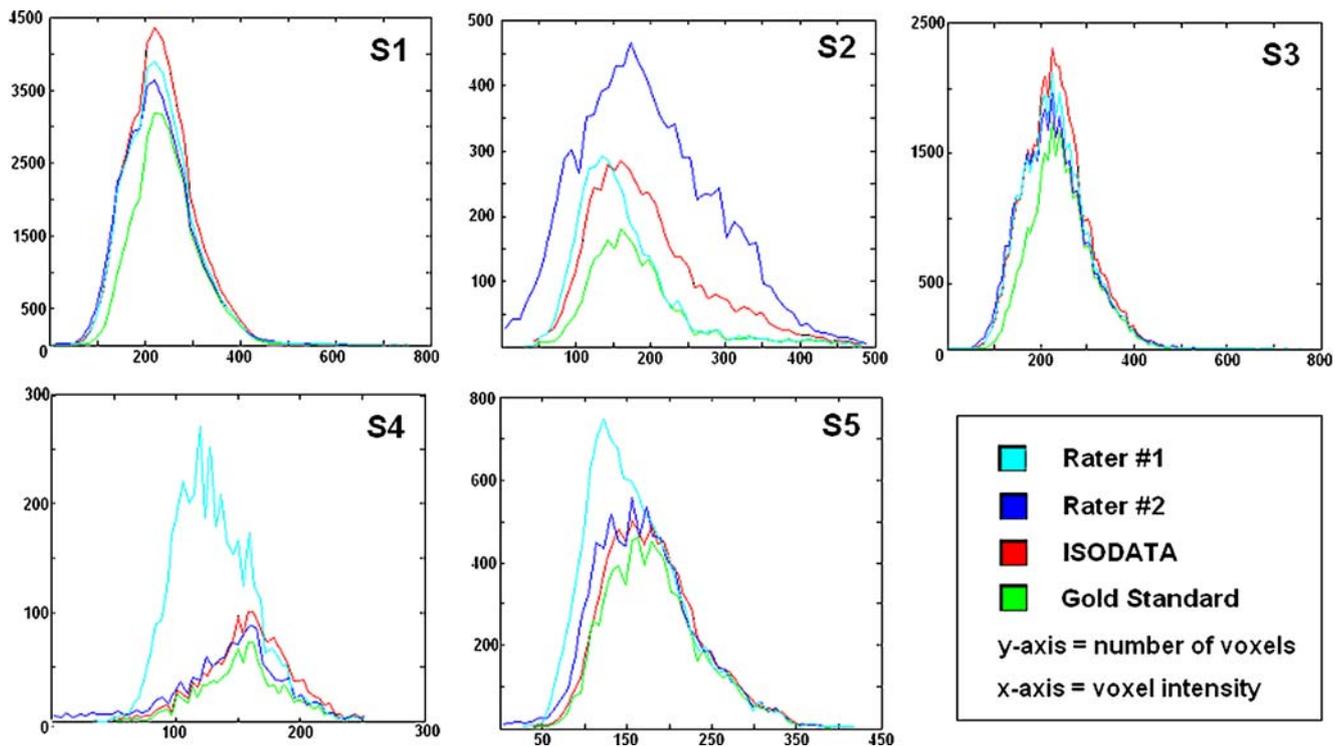
Fig. 4 Demonstrates the distributions in the voxels identified as "lesion" by the four methods (e.g., Expert 1, Expert 2, GS, ISODATA). The subject numbers listed for each distribution correspond to Tables 1 and 2

or gray matter. It is important to note that the lesion overlap analysis indicates that ISODATA was able to characterize distinct lesion sizes, including isolated focal lesions (evident in Fig. 6) and very severe, extensive lesions (evident in Fig. 2b) with comparable efficacy.

The data above indicate that ISODATA maintains excellent sensitivity to brain abnormality, detecting a greater number of hyperintense, or "abnormal" voxels compared to automated procedures (see Fig. 4, Table 1). A primary advantage to this method, however, is that it also maintains specificity regarding tissue type; unlike semi-automated methods, ISODATA provides the opportunity to examine tissue subtypes within lesion clusters. In the current cases of TBI, ISODATA commonly identified three

Table 2 Reveals the r^2 values and error in measurement for ISODATA results for three levels of simulated noise

Subj	# of Slices	5% Noise	15% Noise	25% Noise
1	11	98 (3)	97 (4)	96 (6)
2	4	93 (2)	92 (4)	91 (5)
3	13	94 (2)	92 (3)	90 (5)
4	2	92 (4)	90 (6)	88 (7)
5	8	96 (2)	95 (3)	93 (3)
	38[a]	94.6 (3)	93.2 (4)	91.6 (5)

[a] = total number of slices examined, all other summary values are means.

separable voxel clusters (i.e., lesion classes), that were consistently higher in signal intensity than gray/white voxels in the distribution.

The reliability of ISODATA was established by examining ISODATA results at each voxel after adding three levels of random noise (e.g., 5%, 15%, 25%). This noise was added to the original image over 50 iterations and averaged to produce a new image for each level of random noise. The original voxel without noise was used as a regressor to predict the same voxel after adding one of three levels of white noise; the original voxel cluster (1 though 20) accounted for >90% of the variance in the voxel cluster at all noise levels. These data indicate that ISODATA is a robust technique that provides highly reliable results even in cases of degraded image quality.

Voxel distribution and histogram results

The histogram results presented in Fig. 1 reveal the distribution of voxel intensities for healthy adults compared to an individual with TBI, illustrating the dissimilarity between the normal and abnormal voxel distributions (right tail of the distribution seen in Fig. 1d). The voxel distributions for healthy adults and individuals with TBI were consistently differentiated, yet the current findings also revealed that the MRI signal intensity in "abnormal" voxels varied within groups. That is, even within the group
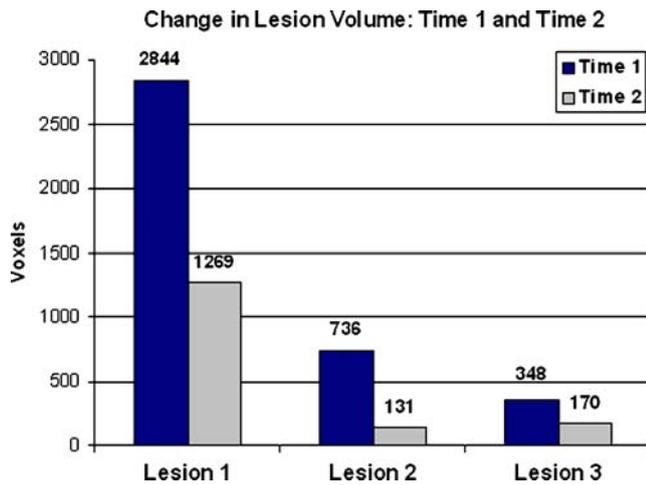
Fig. 5 The average values for the three lesion subtypes identified by ISODATA at Time 1 and Time 2; all lesion subtypes diminish in size between time points

of individuals with TBI, there were small differences in the absolute MRI signal intensity (the x-axis in histogram results) that differentiated tissue classes. Because of this, a rigid cut-off score does not accurately capture the within group differences in the voxel intensities in the brain lesions evident in this sample. The current method capital-

izes upon the flexibility of ISODATA by separating lesion clusters on an image-by-image basis without thresholding; voxel "abnormality" is relative and partially contingent upon the signal intensity of neighboring voxels. This is a distinct advantage to using ISODATA compared to techniques using an absolute threshold.

The distributions presented in Fig. 4 represent those voxels determined to be "lesion" and demonstrate the consistency between ISODATA each of expert raters and the GS. Of note, ISODATA included a greater number of voxels than the GS at virtually every voxel intensity. These data demonstrate the sensitivity of ISODATA in detecting a range of voxel values determined to be "lesion" by semi-automated procedures.

ISODATA and applications

There are several important advantages to the ISODATA method of lesion identification used here. First, this method does not require *a priori* identification of brain lesions or complicated MRI methodology. Because of this, ISODATA is ideal for rapid and reliable lesion quantification of lesions due to TBI using a single clinical FLAIR scan at 1.5 T.

The current findings demonstrate that ISODATA permits whole-brain voxelwise quantification of lesions with sensi-
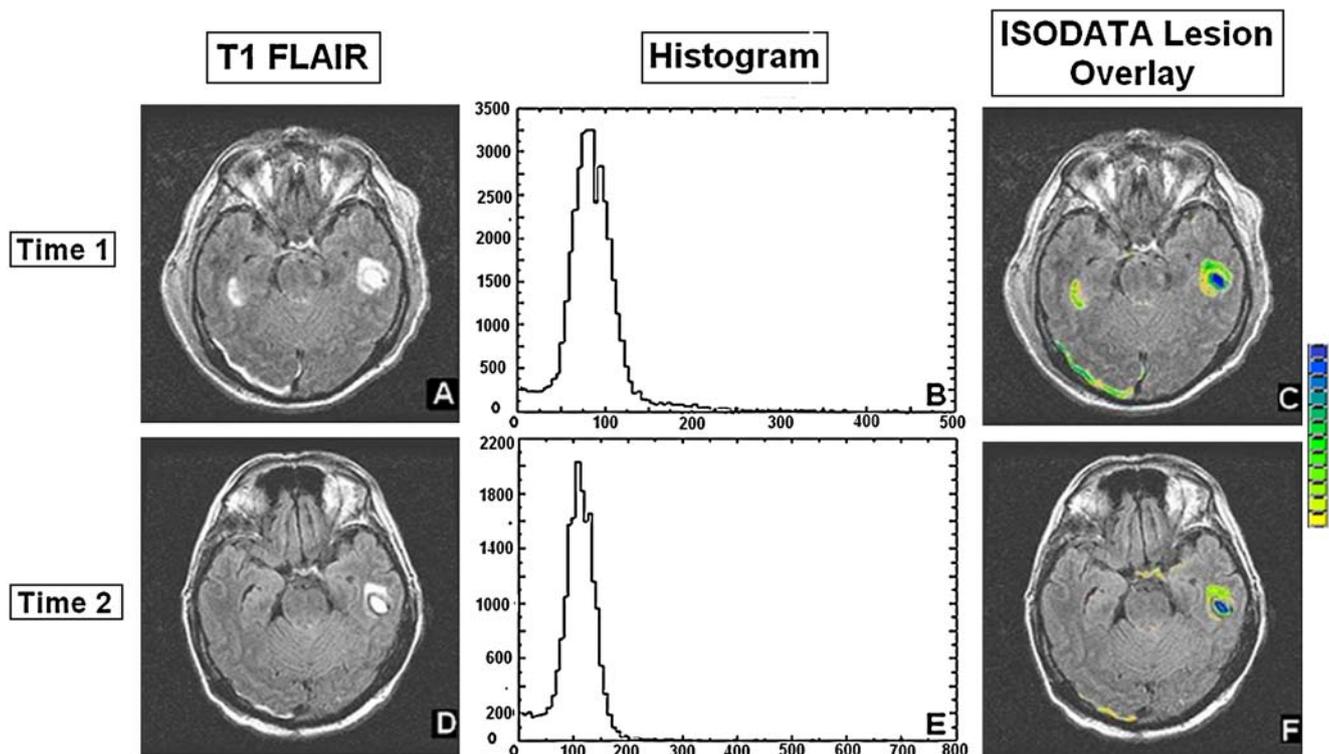
Fig. 6 ISODATA results at separate time points (frames A through F) For both Figures, B and E depict the histogram analyses for each time point and C and F reveal the color-coded areas determined to be lesion by ISODATA. Note, the number of voxels maintaining the highest signal intensity decreases from time point 1 to time point 2, mirroring reduction in the overall lesion size
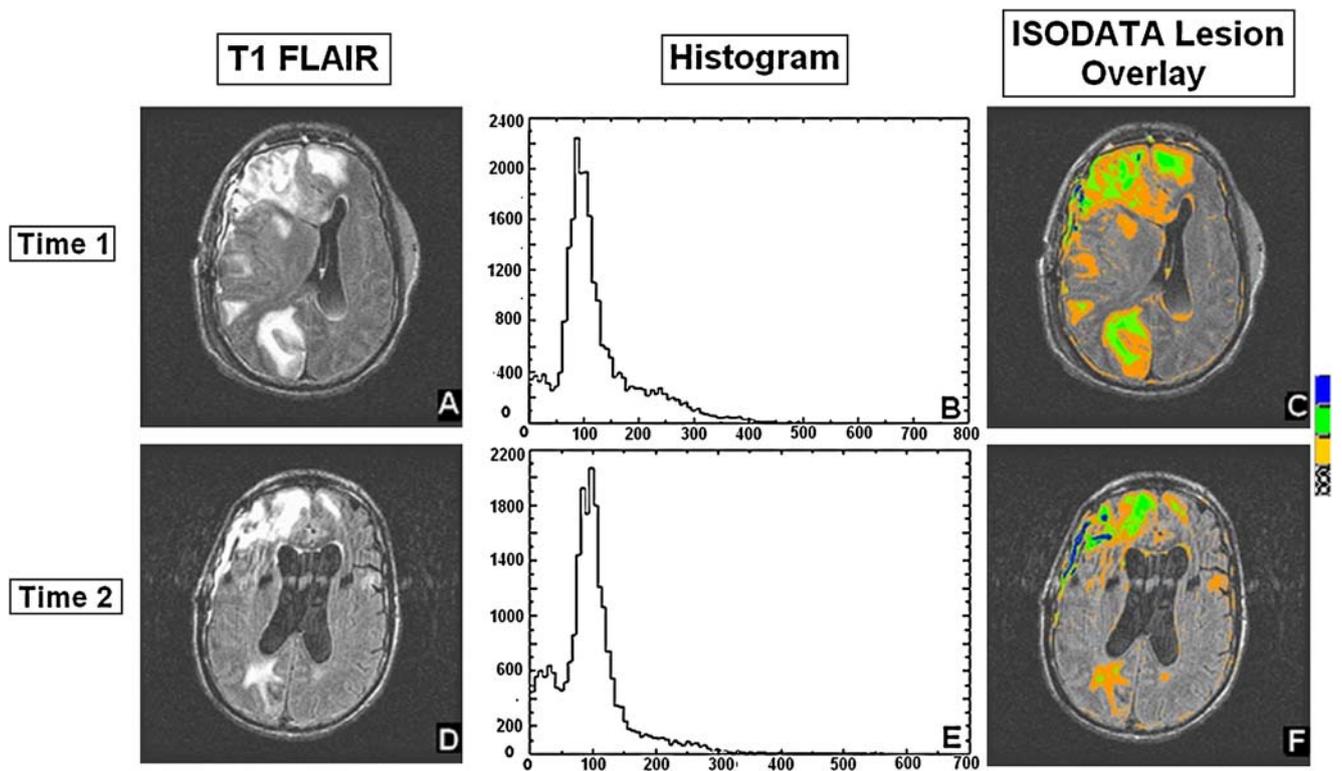
Fig. 7 ISODATA results at separate time points (frames A through F) For both Figures, B and E depict the histogram analyses for each time point and C and F reveal the color-coded areas determined to be lesion by ISODATA. Note, the number of voxels maintaining the highest signal intensity decreases from time point 1 to time point 2, mirroring reduction in the overall lesion size

tivity to alterations in lesion size and extent over time. Over the course of the first few weeks of recovery from neurotrauma, significant lesion resolution is expected and, in the cases presented here, gross changes were apparent on MRI. Based upon ISODATA results, total lesion volume decreased in size for all but one of the slices examined (for average values of the three lesion clusters at each time point see Fig. 4). ISODATA thus permits voxelwise comparisons in dissociable lesion subtypes at separate time points to examine the trajectory of change in distinct lesions subtypes. Moreover, ISODATA may be coupled with other MRI techniques documenting change in metabolism and brain function during recovery.

A final, but important advantage to the ISODATA method is that it affords the ability to differentiate the voxels of highest signal intensity (the primary lesion, or lesion center) and the surrounding peri-lesional areas. In the cases presented here, the secondary and tertiary lesion tissue classes, could reflect intermixed healthy and damaged tissue or areas where loose blood resides over viable neural tissue. Through the use of ISODATA, it is possible to identify these sites of vulnerability and to isolate and monitor the evolution of these lesion clusters independently. This procedure thus identifies borderline, or "at risk" tissue, so that examiners can work to characterize the

influence of TBI on basic physiological parameters such as cerebral blood flow and oxygen extraction fraction in these areas. For example, the influence of identifiable brain lesions following TBI on the BOLD fMRI signal are just beginning to be understood (Hillary and Biswal 2007).

One interesting, and unexpected, outcome of the current study is that the ISODATA procedure was relatively unaffected by head movement. In the second exam for TBI case 2, the subject remained in a coma, but generated very small head movements during data acquisition (see Fig. 7d). This movement resulted in mild image degradation which challenges semi-automated procedures requiring visual inspection and identification of lesion margins. In addition to introducing signal distortion, head movement may increase partial volume effects, permitting MRI signal contributions by both white and gray matter, for example, within the same voxel. In Fig. 7d, the histogram results from the first scan to the second scan did not reveal significant changes in the general shape of the voxel distribution; that is, one expectation of partial volume effects would be a smoothing of the distribution (See Fig. 7b, e). ISODATA thus appears to be unaffected by head movement and, to verify this, future work in our laboratory will examine the influence of image degradation on the performance of this algorithm by introducing

motion-related effects. Again, greater head movement is expected in clinical samples and an automated algorithm that is able to consistently separate clusters in degraded images will have greater utility.

While the current results demonstrate that ISODATA is effective in identifying and quantifying various lesion configurations following TBI, additional analysis with a larger sample are needed to support these observations. Future work using ISODATA might also examine milder forms of TBI and the effects of distinct pathophysiology (e.g., multiple sclerosis, HIV). One important next step will also to be to determine if the lesion subtypes differentially predict behavior (e.g., cognitive, motor, sensory functioning).

Conclusion

In conclusion, the current study adapted and validated an automated lesion quantification method, ISODATA, for rapid identification and quantification of brain lesions following TBI. The ISODATA method used here overlapped significantly with a traditional semi-automated approach and was sensitive to diminishing lesion size during the first month of recovery from moderate and severe TBI. The ISODATA method provides researchers with a tool capable of isolating areas of lesion and differentiating distinct clusters within each lesion area based upon MRI signal intensity. The current data also demonstrate that ISODATA maintains sensitivity to changes in lesion size during recovery. Finally, by knowing the parameters of any given lesion space, investigators may test hypotheses regarding the relationship between brain lesion sites and structural and functional changes throughout the brain by using ISODATA in conjunction with advanced MRI methods.

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