Voxel-based morphometry of brain SPECT can detect the presence of active central nervous system involvement in systemic lupus erythematosus


Objective. To determine the value of voxel-based morphometry (VBM) of brain SPECT (single-photon emission computed tomography) images (BSI) in discriminating active central nervous system (CNS) manifestations in systemic lupus erythematosus (SLE) patients.

Patients and Methods. Forty SLE patients (mean age 33 yrs) and 33 normal volunteers were submitted to BSI. SLE patients were screened for the presence of CNS involvement following the American College of Rheumatology (ACR) case definition. Patients with CNS infections, uraemia, diabetes and previous ischaemic or haemorrhagic stroke were excluded. Magnetic resonance imaging (MRI) scans were obtained in a 2T scanner (Elscent Prestige) with T1- and T2-weighted images. BSI were performed after injection of 1110 MBq (30 mCi) of 99mTc-ECD (ethyl-cysteinate-dimer). BSI were analysed using the statistical parametric mapping. After normalization, segmentation and smoothing the groups of SLE patients with active and inactive CNS manifestations and healthy volunteers were compared using VBM. Post-processed images were compared voxel-by-voxel using t-test in order to determine differences of intensity between groups. This analysis included grand mean scaling, proportional threshold masking (set to 0.4) and implicit masking. A P-value of 0.001 and cluster size of 32 were taken into consideration.

Results. VBM analyses of BSI did not show any differences between SLE patients with inactive CNS involvement and normal controls. However, the group of SLE patients with active CNS involvement had a global hypoperfusion, more intense in the frontal, dorsolateral and medial temporal lobe when compared with SLE patients without CNS involvement (P = 0.001) and healthy volunteers (P = 0.001).

Conclusion. VBM of BSI is a useful and objective method for detecting perfusion abnormalities in SLE patients, which is indicative of active CNS involvement. However, it is not helpful in differentiating the clinical sub-types of CNS involvement according to the ACR classification.

Key words: SPECT, VBM, SLE.

Introduction

Central nervous system (CNS) involvement is seen in as many as 11–60% of systemic lupus erythematosus (SLE) patients and may cause transient neurological manifestations or chronic brain injury [1, 2]. The diagnosis of CNS involvement of SLE is difficult because of the need to differentiate primary from secondary causes of neurological involvement such as CNS infections and metabolic encephalopathy. In addition, the absence of reliable serum markers and an ideal imaging modality increases this difficulty [3].

Magnetic resonance imaging (MRI) is the preferred anatomic imaging modality [3]. MRI is more likely to show abnormalities if there are focal neurological deficits, seizures, chronic cognitive dysfunction or the antiphospholipid syndrome. However, in many patients with obvious CNS involvement, MRI may not show abnormalities, especially in patients with affective disorders, confusional states or headaches [3, 4].

Another drawback of MRI is the difficulty in differentiating lesions of active CNS manifestations from old lesions [5, 6]. MRI frequently (25–50%) reveals chronic lesions in patients with and without active disease, and the incidence of these lesions increases with age, disease severity and past history of CNS involvement [3, 6, 7]. Therefore, techniques for detecting functional brain abnormalities may be useful. Several functional techniques have been used in SLE, including positron emission tomography (PET), magnetic resonance spectroscopy (MRS), and single-photon emission computed tomography (SPECT). PET with 18-fluoro-2-deoxyglucose (FDG) shows cerebral involvement in patients with NP-SLE who have no morphological changes detectable by CT and MRI. PET is considered to be a sensitive and reliable method for evaluating SLE patients with CNS involvement. However, it is not yet established in routine clinical use because it is expensive and not available in most hospitals [8]. MRS is another functional method that may be used for detecting CNS manifestations in SLE. Although it...
is more frequently used than PET, most studies use single-voxel MRS, and so only a predetermined volume of the brain may be analysed [9]. Multi-voxel MRS is still rare in clinical practice [9]. Therefore, brain SPECT may be an alternative functional method for detecting functional abnormalities in CNS manifestations in clinical practice, providing important clinical information by imaging regional blood flow changes. Several studies used brain SPECT images (BSI) to investigate patients with SLE [3, 8, 10–20]. Hypoperfusion, suggesting decreased regional blood flow, were identified in SLE patients with neuropsychiatric manifestations [10, 12–17], although others have also found perfusion abnormalities in SLE patients without neuropsychiatric manifestations [18–20]. Most studies use visual analysis with regional quantification of perfusion abnormalities. Semi-quantitative analysis of BSI have increased diagnostic yield of BSI in other diseases [21–23]. Statistical parametric mapping (SPM) is an increasingly established form of neuroimaging analysis to detect statistically significant differences in spatially normalized images on a voxel-by-voxel basis [24–26]. The use of SPM eliminates observer subjectivity inherent in visual analysis [27–29]. The purpose of this study was to determine the value of voxel-based morphometry (VBM) of brain SPECT images (BSI) in discriminating active CNS manifestations in SLE patients.

Subjects and method

Subjects

Sixty consecutive SLE patients fulfilling four or more criteria for classification of SLE [30] with CNS involvement were invited to participate in the study. All patients had active or past history of CNS involvement as defined by the American College of Rheumatology (ACR) case definition [31]. Active CNS disease, as defined by the new onset or persistence of a CNS manifestation at the time of examination, was identified in 27 patients. The remaining 33 patients had inactive (past history) CNS involvement. Patients were followed-up prospectively in the Rheumatology Unit of the State University of Campinas (UNICAMP).

We excluded patients with associated clinical conditions that could cause cerebral atrophy, such as stroke (10 patients), arterial hypertension (one patient), diabetes mellitus, alcohol and drug abuse and malignancy. Patients satisfying the ACR criteria for rheumatoid arthritis, systemic sclerosis, Sjögren syndrome (two patients) or other connective tissue disease and with drug-induced SLE were also excluded.

Total dose of corticosteroids and other immunosuppressant medications used since the onset of disease were estimated using the data obtained by careful review of the medical charts. Seven patients with incomplete charts were excluded from the analysis.

Therefore, 40 patients (20 with active and 20 with inactive CNS manifestations) were submitted to BSI. The control group consisted of 50 healthy age- and sex-matched volunteers.

This study was approved by the Ethical Committee of our institution and informed, written consent was obtained from each subject.

Clinical, serological and treatment features of SLE patients

Clinical manifestations. Data on gender and age at disease onset and disease duration were collected for each patient. Disease duration was defined as the initial manifestation clearly attributable to SLE until the day of BSI acquisition. All clinical manifestations and laboratory test findings were recorded according to ACR criteria [30, 31]. Disease activity was measured in all visits using the systemic lupus erythematosus disease activity index (SLEDAI), which is a standardized score for SLE patients and includes 24 items [32]. The SLEDAI score is calculated by summing the predetermined weights for the items that are present. Items that are life-threatening have higher weights, with possible scores ranging from 0 to 105. Cumulative organ damage was analysed by validated damage index (SLICC/ACR-DI) [33]. SLICC/ACR-DI is an unweighted index composed of 41 items grouped in 12 domains, with a maximum possible score of 47. As previously established, damage was considered when the irreversible lesions were present for at least 6 months unrelated to active inflammation and had occurred after SLE diagnosis [33].

CNS involvement. A complete neurological examination, as well as cognitive and psychiatric charts, were prospectively applied to all patients during their clinical visit in order to identify active CNS involvement [31]. Mini Mental State Examination [34] was applied to all participants. All patients were submitted to a battery of standardized neuropsychological tests in order to screen for possible impairment in one or more of the subsequent cognitive domains: simple attention, complex attention, memory, visuospatial processing, language, reasoning/problem solving, psychomotor speed and executive functions [35–38]. These tests have not been validated for SLE patients, but are widely used for patients with CNS disorders in clinical practice and research. The individual test results were converted into standard scores, which were compared with the available normative data [35–38]. Regarding any of the eight cognitive domains, subjects with a total score of 2 s.d. below the normative value were considered to be impaired. Cognitive dysfunction was classified as mild if there were deficits in less than three dimensions, as moderate if there were deficits in three or four dimensions and as severe if there were deficits in at least five dimensions [38, 39].

Assessment of depression was based on clinical interview and the Beck Depression Inventory (BDI) [40, 41]. On BDI, scores from 10 to 17 were considered to indicate mild depression, from 18 to 24 moderate depression and greater than 24 severe depression. Anxiety was evaluated by anxiety through the Hospital Anxiety and Depression scale [42]. The presence of psychosis was determined through the Brief Psychiatric Rating Scale (bBPRS) [43].

For past history of CNS involvement we reviewed the medical charts of patients.

Laboratory features. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using mouse liver as the substrate, and were regarded as positive if higher than 1:40. Anti-double-stranded DNA (dsDNA) antibodies were determined by indirect immunofluorescence using Chlridiida as substrate, and were considered positive if higher than 1:10. Precipitating antibodies to extractable nuclear antigens (ENA), including Ro (SSA), La (SSB) and Sm were detected by immunodiffusion and/or microhaemagglutination. Anticardiolipin antibodies (aCL) of the IgG and IgM isotypes were measured by the ELISA method [44]. Lupus anticoagulant (LA) activity was detected by coagulation assays in platelet-free plasma obtained by double centrifugation, following the recommendation of the subcommittee on LA of the Scientific and Standardization Committee of the International Society of Thrombosis and Homeostasis [45].

BSI acquisition. After completing inclusion and exclusion criteria, 40 patients (20 with active and 20 with inactive CNS manifestations) were required to remain resting in a dark, quiet room for 15 min, with a permanent intravenous access through a butterfly connected to a catheter with saline solution. While at rest, 1110 MBq (30 mCi) of 99mTc-ECD (ethyl-cysteinate-dimer) was injected. The patients remained resting for another 10 min. BSI was performed in a computed scintillation camera with a fan-beam collimator. Sixty images were acquired in a 64 × 64 matrix, every 6°, in a total of 360°. Raw data were reconstructed by filtered backprojection, and attenuation correction was
performed using Chang’s method with a 0.115 attenuation coefficient.

BSI analysis. The reconstructed BSI were converted into Analyze format using MRICro software (www.mricro.com). Voxel-based analysis was performed using SPM2 (Wellcome Department of Cognitive Neurology, London).

To allow group comparisons, the size and shape of each individual’s scan were normalized to stereotaxic space by estimating the optimum 12-parameter affine transformation [46–48]. The $^{99m}$Tc-ECD uptake was standardized to the mean global uptake using a proportional scaling. At this point, standard VBM preprocessing steps include segmentation of brain tissues, but because BSI signals are acquired from radio tracers dispersed in blood flow and most of blood vessels are in the GM portion of brain, this step was not necessary and is not recommended for nuclear medicine images to avoid spatial resolution worsening. The images were subsequently smoothed by convolving its voxels with an isotropic Gaussian kernel (IGK) of 10 mm in order to minimize border effects caused by gyril inter-individual variability and create images that can be spatially compared with a good correspondence of analogous tissues.

MRI acquisition protocol and analysis. MRI scans were obtained in a 2T Elscint Prestige scanner. T1-weighted gradient-echo sequence with 1 mm thickness (TR = 22 ms, TE = 9 ms, flip angle = 35° and matrix = 256 x 220) was used for voxel-based morphometry (VBM) analysis. Images were normalized to the standard space using 12 linear parameters and 7 x 8 x 7 nonlinear basis functions, using a brain mask. Spatially normalized images were re-sliced to isotropic voxels of 1.5 mm and underwent segmentation of white and gray matter. The images were smoothed by convolution with an IGK of 10 mm in order to minimize inter-individual gyral variability. The resulting images were then compared voxel-by-voxel by using t-test to determine differences in gray matter between patients with active and inactive CNS involvement and controls using statistical parametric mapping (SPM 2). This analysis included grand mean scaling and proportional threshold masking (set to 0.4) and implicit masking. A $P$-value of 0.001 was taken into analysis, and the minimum cluster size taken into account was 32.

Statistical analysis. Group differences for age were assessed using one-way ANOVA, and the gender distribution was evaluated with the chi-square test.

The statistical analysis of the normalized and smoothed BSI data was performed using the SPM2. Statistical analysis was performed by comparing both groups of patients (with and without CNS manifestations) with the control group. It was also performed a comparison between the group of patients with CNS manifestation and the group of patients without manifestation. These comparisons between these groups were performed using a non-paired two-sample t-test. Only voxels with signal intensity above a threshold of 0.4 were entered in each analysis. It was used with a $P$-value of 0.001 with false discovery rate (FDR) and a cluster size of 32. The tool FDRs minimize errors from multiple comparisons and eliminates, from the final statistical $t$-map, all values with a probability of being a false positive discovery. This step is important since the natural variability can produce false discoveries and lead to incorrect results [49]. The areas of hypoperfusion on BSI were compared with areas of reduced voxel number (atrophy) on MRI in order to determine if the hypoperfusion is due to cerebral atrophy in SLE patients.

In order to determine the relationship between specific clinical manifestation and pattern of reduced blood flow assessment, multiple regression was used.

Results

Demographic data

We included 40 SLE patients with mean age of 33.3 yrs (range 18–45 yrs, S.D. = 12.46). Thirty-eight were women. The mean duration of disease was 31.5 months (range 1–150, S.D. = 58.50) (Table 1).

The control group consisted of 33 healthy volunteers (29 women and 4 men) with mean age 30.6 yrs (range 20–53 yrs; S.D. = 8.65 yrs).

Clinical, laboratory and treatment features

Clinical features are summarized in Table 2. Mean SLEDAI scores was 4.7 points (S.D. = 0.74). Mean SLICC score was 3.9 (S.D. = 2.3) (Table 1). Of 48 CNS manifestations observed in SLE patients, 27 manifestations were active in 20 patients (Table 3). Although headache is the most frequently observed CNS manifestation, it occurred isolated only in two patients with inactive CNS manifestations. The other patients had other CNS manifestations, especially cognitive dysfunction, associated.

All patients were on corticosteroid dose, with doses ranging from 5 to 60 mg/day. Fifteen patients were on chloroquine and eight patients (three with active and five with inactive CNS manifestations) were receiving azathioprine.

MRI findings

Areas of abnormal T2 signal, identified as small white matter lesions, were observed in 10 (25%) patients. Five of these patients had cognitive impairment, four had seizures and one patient had aseptic meningitis. All patients with MRI abnormalities had active (four patients) or past history (six patients) of CNS manifestations. On visual analysis, no further abnormalities

### Table 1. Demographic characteristics in groups

<table>
<thead>
<tr>
<th>Data</th>
<th>SLE with active CNS disease</th>
<th>SLE with past history of CNS disease</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± S.D. yrs)</td>
<td>32.4 ± 11.8</td>
<td>32.6 ± 13.1</td>
<td>30.6 ± 8.6</td>
</tr>
<tr>
<td>Female: male ratio</td>
<td>18/2</td>
<td>20/0</td>
<td>29/4</td>
</tr>
<tr>
<td>Disease duration (mean months ± S.D.)</td>
<td>30.2 ± 10.2</td>
<td>33 ± 8.4</td>
<td>–</td>
</tr>
<tr>
<td>SLEDAI (mean ± S.D.)</td>
<td>5.1 ± 0.6</td>
<td>4.8 ± 0.9</td>
<td>–</td>
</tr>
<tr>
<td>SLICC (mean ± S.D.)</td>
<td>4.1 ± 1.5</td>
<td>3.9 ± 1.9</td>
<td>–</td>
</tr>
</tbody>
</table>

*$P > 0.05$ in all comparisons.

### Table 2. Clinical manifestations in SLE patients with active and inactive CNS involvement

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>SLE patients with active CNS involvement, n (%)</th>
<th>SLE patients with inactive CNS involvement, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>16 (80)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Avascular necrosis</td>
<td>1 (5)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>6 (30)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Fever</td>
<td>18 (90)</td>
<td>18 (20)</td>
</tr>
<tr>
<td>Hemolytic anaemia</td>
<td>4 (20)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>10 (50)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Malar rash</td>
<td>12 (60.0)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>6 (30)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Oral Ulcers</td>
<td>4 (20)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>14 (70)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>6 (30)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Serositis</td>
<td>7 (35)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3 (15)</td>
<td>4 (20)</td>
</tr>
</tbody>
</table>
perfusion than patients with inactive CNS involvement (Fig. 1). These perfusion abnormalities occurred in areas without intense T2 signals on MRI and BSI hypoperfusion (P < 0.001). Colour scale indicates standard deviation from controls.

were detected. Applying VBM to MRI, we observed that SLE patients had a reduced voxel volumes in frontal, dorsolateral and medial temporal lobe when compared with controls (P < 0.001). However, there was no difference in gray matter concentration between patients with inactive and active CNS SLE manifestations (P > 0.005).

Brain SPECT images

We found a significant hypoperfusion, especially in frontal (P < 0.001), parietal (P < 0.001) and medial temporal lobes (P < 0.001), in patients with active CNS involvement when compared with patients with past history of CNS involvement, as well as when comparing SLE patients with active CNS manifestations to healthy volunteers (P < 0.001) (Table 4, Fig. 1). These perfusion abnormalities occurred in areas without structural abnormalities on MRI. No difference between SLE patients without CNS manifestations and healthy volunteers was observed (P > 0.05).

No relation between a specific clinical manifestation, active or inactive, and pattern of reduced blood flow was observed (P = 0.45). There was no relationship between areas of hyperintense T2 signals on MRI and BSI hypoperfusion (P > 0.05).

Discussion

This is the first study using VBM analysis of SPECT images in SLE. Furthermore, using this method, we were able to differentiate active from inactive CNS manifestations in SLE patients.

Table 3. CNS manifestations in patients with active CNS involvement

<table>
<thead>
<tr>
<th>CNS manifestations</th>
<th>CNS involvement, n (%)</th>
<th>Active CNS involvement, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>10 (50)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Seizures</td>
<td>4 (20)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Acute confusional state</td>
<td>4 (20)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>2 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Myelopathy</td>
<td>2 (10)</td>
<td>0</td>
</tr>
<tr>
<td>Aseptic meningitis</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Movement disorder</td>
<td>1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>13 (65)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>5 (25)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Mood disorder</td>
<td>6 (30)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Total number of events</td>
<td>48</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 4. Brain sites where patients with active CNS involvement have less perfusion than patients with inactive CNS involvement

<table>
<thead>
<tr>
<th>Spatial coordinates</th>
<th>Anatomical location</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>−42</td>
<td>−63</td>
</tr>
<tr>
<td>47</td>
<td>−65</td>
</tr>
<tr>
<td>−39</td>
<td>−71</td>
</tr>
<tr>
<td>−50</td>
<td>−71</td>
</tr>
<tr>
<td>−48</td>
<td>−17</td>
</tr>
<tr>
<td>−50</td>
<td>−26</td>
</tr>
<tr>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>−15</td>
<td>45</td>
</tr>
</tbody>
</table>

*Height threshold: T = 3.50, cluster ≥ 20 voxels, FDR corrected (P < 0.05).

Discussion

This is the first study using VBM analysis of SPECT images in SLE. Furthermore, using this method, we were able to differentiate active from inactive CNS manifestations in SLE patients.

This method does not have the subjectivity inherent in visual analysis. SPM has been applied to BSI in several studies [27–29].

SPECT scanning has been used in the assessment of CNS involvement in SLE [3, 8, 10–20, 50–61] and has proved highly sensitive, detecting abnormalities in up to 90% of patients with clinical neuropsychiatric involvement [10, 12–17, 51–53]. However, SPECT has low specificity, and abnormalities are also seen in up to 20% of patients without CNS involvement [18–20]. In our study, using group analysis, we were able to detect perfusion changes only in patients with active CNS involvement. Our study revealed that patients with active CNS involvement had a global hypoperfusion, especially in the frontal, parietal and medial temporal regions when compared with SLE patients with inactive CNS involvement and controls. We did not observe a difference between patients with inactive CNS involvement and healthy controls, suggesting that the hypoperfusion was directly related to disease activity in the CNS. Comparing MRI of patients with active CNS to patients with inactive CNS, we could also demonstrate that these abnormalities are not due to cerebral atrophy in these regions.

Using the VBM method, we compared voxel-by-voxel the perfusion pattern of SLE patients with and without CNS involvement and were able to detect statistically difference in patients with active CNS involvement. These changes were not evident on visual analysis. However, it is not helpful in differentiating the different clinical sub-types of CNS involvement.

BSI hypoperfusion abnormalities have been reported in the middle cerebral artery distribution, parietal (65–80%), frontal (57–65%) and temporal lobes (46–57%) in SLE patients. Basal ganglia hypoperfusion is much less common (12–30%) [15, 59]. In neuropathological analysis, the most frequent findings in brains of SLE patients are multiple microinfarcts, which are related to vasculopathy with small vessels presenting thickening of the intima and fibrinoid degeneration [58]. Therefore, in patients with CNS manifestations, decrease cerebral blood flow due to loss...
of cerebral perfusion reserve occurs earlier than detected on structural MRI. This explains why our patients with active CNS manifestations presented hypoperfusion on BSI images. In agreement with previous data [10, 13, 15], we did not find a relationship between the type of CNS manifestations and the pattern of perfusion abnormalities. Thus, BSI scanning may be used mainly to support a clinical diagnosis of active neuropsychiatric involvement as a syndrome and not to differentiate between different types of manifestations. Perhaps the inclusion of a greater number of individual manifestations could differentiate different patterns of hypoperfusion on BSI. In order to determine if hypoperfusion could be secondary to cerebral atrophy, we analysed the MRI data of patients and controls. Although we observed a statistical difference in relation to gray matter volume reduction in patients when compared with controls, there was no difference in gray matter volume between patients with active and inactive CNS involvement. These findings support the idea that hypoperfusion could be secondary to disease activity in the CNS and not only due to the cortical atrophy.

The absence of SLE patients with quiescent SLE and SLE patients with active disease without CNS involvement is a limitation of this study. Further studies are necessary to determine the relationships between structural and functional abnormalities in these groups of patients.

Because functional abnormalities may precede anatomic abnormalities, perfusion and metabolic studies employing BSI are complementary to MRI in diagnosing CNS involvement. Qualitative analysis of BSI has an interobserver variability and the importance of combining neuroimaging studies has been emphasized in order to assess both brain structure and function.

### Key message

- VBM of BSI is a useful and objective method for detecting perfusion abnormalities.
- VBM was able to differentiate active from inactive CNS manifestations in SLE patients.
- It is not helpful in differentiating the clinical sub-types of CNS involvement.

The authors have declared no conflicts of interest.

### References

47. S. Appenzeller