Supplemental Data

Scalp-EEG recording and video-EEG monitoring

Video-EEG monitoring was performed in the epilepsy unit for 5 days and antiepileptic drugs were reduced where necessary to facilitate seizure occurrence. Ictal scalp recordings were obtained using scalp electrodes placed according to the international 10/20 system, with additional fronto-temporal electrodes following the 10/10 system. Video-EEG monitoring was performed using Deltamed equipment (Natus Medical Incorporated, San Carlos, CA, USA.) with 128 recording channels and was interpreted by an epileptologist from the epilepsy unit at our centre who had experience with pediatric EEG.

MRI

MRI was performed using a 1.5 T unit (Signa Exite; GE Healthcare, Milwaukee, WI, U.S.A.) with a specific epilepsy protocol that included the following sequences: sagittal T1-weighted inversion recovery (repetition time [TR] 2,291 ms, echo time [TE] 16 ms, inversion time [TI] 750 ms, 5-mm slice thickness); coronal proton density (PD)/T2 dual fast spin echo (TR 5,240 ms, TE 29/87 ms, 3-mm slice thickness); coronal 3D magnetization prepared rapid acquisition gradient echo (TR 13.3 ms, TE 5.6 ms, 1.4 mm slice thickness); coronal fluid-attenuated inversion recovery (FLAIR) (TR 9,002 ms, TE 131 ms, and T1 2,000 ms, 4 mm slice thickness); and axial PD/T2 dual fast spin echo (TR 3,300 ms; TE 22/88 ms, 5 mm slice thickness). All coronal sequences were acquired parallel to the long axis of the hippocampus and all sequences covered the full brain.

SPECT and SISCOM

Due to programmatic reasons, all patients underwent video-EEG for at least 4 days as most pediatric patients require sedation during the acquisition of the ictal-SPECT. All patients were admitted on Mondays and antiepileptic drugs were reduced gradually when necessary to ensure seizure will be present on Thursday. Ictal SPECT is scheduled on Thursdays because it is the only day the anesthesiology team is available to perform sedation in case needed. Ictal and interictal SPECTs with 99mTc-ethyl cysteinate dimer (ECD) were performed on the first 38 patients, but due to the pharmacological discontinuation of ECD in July 2011, 99mTc-hexamethylpropyleneamine-oxime (HMPAO) was used as radiotracer for the remaining 16 ictal and interictal SPECTs.

The appropriate patient dose was calculated based on the patient's weight as well as the time lag between dose calibration and seizure onset, extrapolating from the standard adult dose of 740 MBq (20 mCi). For this purpose, the syringe volume to be administrated was adjusted according to ^{99m}Tc decay; always bearing in mind the radiochemical stability of HMPAO (5 h) and ECD (10 h).

SPECT studies were acquired within two hours of radioisotope injection and following the same protocol using a dual-head SPECT imaging system (Infinia Hawkeye 4; GE Healthcare Milwaukee, WI, USA) with a specific epilepsy protocol that comprised the use of a low-energy high-resolution parallel-hole collimator. Using a rotation radius of 14 cm, 120 projections were acquired over 360º at

40s/projection in a 128x128 matrix with a pixel size of 3.32x3.32 mm2. Images were reconstructed using a filtered back projection algorithm with a Butterworth filter (fc=0.42; order 0.52) of the same matrix size. Sedation was used in selected cases.

SISCOM methodology (*1*) was performed using FocusDET, a software toolbox for SISCOM analysis developed within a CIBER BBN project (*2*). The main steps to perform SISCOM analysis with FocusDET are: 1) Registration of Ictal and Interictal SPECT studies by using a rigid body transformation with a local correlation coefficient as a cost function (*3*); 2) Intensity normalization and subtraction of Ictal and Interictal SPECT Studies; 3) Generation of a MRI mask to extract the brain region (*4*); 4) Registration of SPECT and MRI Studies with a multi-resolution rigid registration scheme (*5*) and finally; 5) Fusion of epileptogenic focus information with MRI by segmenting the subtraction image to show only those voxels with values greater than a selected number of standard deviations of the subtraction image distribution above the mean.

Abnormal findings were defined purely on visual assessment as a hypoperfused area in interictal SPECT and a hyperperfused area in ictal SPECT. SISCOM images were obtained by a subtraction of the interictal study to the ictal SPECT co-registered to MRI and abnormal findings were defined as brain areas greater than two standard deviations (*6,7*) above mean activity of substraction image considering all voxels.

FDG-PET

FDG-PET images were acquired in 3D mode using PET/CT equipment (Biograph; Siemens, Erlangen, Germany) with a specific epilepsy protocol: Images were acquired 40 min after intravenous injection of approximately 5 MBq/kg of FDG, using a standard 11 min routine (1 min for transmission and 10 min for emission). Patients were silent and resting in a dimly lit room during the 40 min following FDG administration, after which 35 tomographic, attenuation-corrected brain sections (oblique, sagittal, and coronal) were obtained (2.47 mm slice thickness). Reconstruction was performed with ordered subset expectation maximization (OSEM) algorithm (16 subsets and six iterations) with a matrix of 128 x 128 x 64 and 2.6 x 2.6 x 2.4 mm^3 voxel size.

FDG-PET studies were registered with MRI using registration algorithms in FocusDET, with a multi-resolution rigid registration scheme. Fusion images were interpreted visually with a transparency of FDG-PET over the MRI that could be freely modified from 0-100%. Hypometabolic brain areas shown in FDG-PET were considered to be the SF.

Supplemental References

1. O'Brien TJ, O'Connor MK, Mullan BP, et al. Subtraction ictal SPET co-registered to MRI in partial epilepsy: description and technical validation of the method with phantom and patient studies. *Nucl Med Commun*. 1998;19(1):31–45.

2. Marti Fuster B, Esteban O, Planes X, et al. FocusDET, a new toolbox for SISCOM analysis. Evaluation of the registration accuracy using Monte Carlo simulation. *Neuroinformatics*. 2013;11(1):77–89.

3. Ros D, Espinosa M, Setoain JF, Falcon C, Lomena FJ, Pavia J. Evaluation of algorithms for the registration of 99Tcm-HMPAO brain SPET studies. *Nucl Med Commun*. 1999;20(3):227–236.

4. Wollny G, Ledesma-Carbayo MJ, Santos A. MIA—A toolbox for medical image analysis. *Work open-source Med image Anal software 2012, Barcelona, Spain mia.sourceforge.net*.

5. Studholme C, Hill DL, Hawkes DJ. Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures. *Med Phys*. 1997;24(1):25–35.

6. Koo CW, Devinsky O, Hari K, Balasny J, Noz ME, Kramer EL. Stratifying differences on ictal/interictal subtraction SPECT images. *Epilepsia*. 2003;44(3):379–86.

7. Newey CR, Wong C, Wang ZI, Chen X, Wu G, Alexopoulos A V. Optimizing SPECT SISCOM analysis to localize seizure-onset zone by using varying z scores. *Epilepsia*. 2013;54(5):793–800.