# Towards prognostic biomarkers from BOLD fluctuations to differentiate a first epileptic seizure from new-onset epilepsy

FULL-LENGTH ORIGINAL RESEARCH

# Towards prognostic biomarkers from BOLD fluctuations to differentiate a first epileptic seizure from new-onset epilepsy

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## Summary

**Objective:** **The diagnosis of epilepsy cannot be reliably made prior to a patient's second seizure in most cases. Therefore, adequate diagnostic tools are needed to differentiate subjects with a first seizure from those with a seizure preceding the onset of epilepsy. The objective was to explore spontaneous blood oxygen level–dependent (BOLD) fluctuations in subjects with a first-ever seizure and patients with new-onset epilepsy (NOE), and to find characteristic biomarkers for seizure recurrence after the first seizure.**

**Methods:** **We examined 17 first-seizure subjects, 19 patients with new-onset epilepsy (NOE), and 18 healthy controls. All subjects underwent clinical investigation and received electroencephalography and resting-state functional magnetic resonance imaging (MRI). The BOLD time series were analyzed in terms of regional homogeneity (ReHo) and fractional amplitude of low-frequency fluctuations (fALFFs).**

**Results:** **We found significantly stronger amplitudes (higher fALFFs) in patients with NOE relative to first-seizure subjects and healthy controls. The frequency range of 73–198 mHz (slow-3 subband) appeared most useful for discriminating patients with NOE from first-seizure subjects. The ReHo measure did not show any significant differences.**

**Significance:** **The fALFF appears to be a noninvasive measure that characterizes spontaneous BOLD fluctuations and shows stronger amplitudes in the slow-3 subband of patients with NOE relative first-seizure subjects and healthy controls. A larger study population with follow-up is required to determine whether fALFF holds promise as a potential biomarker for identifying subjects at increased risk to develop epilepsy.**

**KEY WORDS:** **Functional magnetic resonance imaging, BOLD time series, Low frequency oscillations, Regional homogeneity, New-onset epilepsy, First-seizure.**

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## Key Points

- New-onset epilepsy is characterized by aberrant BOLD low-frequency oscillations
- Oscillation amplitudes in new-onset epilepsy were higher in the slow-3 frequency subband than after a first seizure
- Low-frequency oscillations are hypothesized as a potential biomarker to identify seizure recurrence

Approximately 4% of people will have one or more (non-febrile) seizures in their lifetime,[1] whereas only 1% will end up having the diagnosis of epilepsy.[2] Because the initial clinical assessment only seldom provides a definite diagnosis,[3] this leads to uncertainty about the prognosis[4] and treatment initiation. To improve this assessment, adequate diagnostic tools are needed to discriminate between people with a first-ever seizure event from those with a seizure that precedes the onset of epilepsy.[5] Promising diagnostic methods are based on detecting abnormalities in the dynamics of recorded brain signals.[6]

Electroencephalography (EEG) applications have already been described in the literature for the prediction of individuals that might develop epilepsy after a first seizure. These studies have shown that EEG along with clinical investigation could be used to assess which patients might develop epilepsy (misdiagnosis up to 30%).[3,5,7,8] For these EEG studies, sample frequencies in the range of 0.5–4 Hz and 1–20 Hz (i.e. slow-1, delta and theta subbands) were explored.

An alternative to EEG is provided by resting-state functional magnetic resonance imaging (MRI), which explores the spontaneous blood oxygen level–dependent (BOLD) fluctuations. Previously, it was shown that patients with epilepsy (temporal lobe or idiopathic generalized) reveal abnormal BOLD fluctuations in comparison to healthy controls.[9,10] In particular, by using concepts such as regional homogeneity (ReHo) and low-frequency oscillations, it was possible to detect differences between patients with epilepsy and healthy controls. The ReHo analysis method was proposed by Zang et al.[11] and uses the Kendall's coefficient of concordance to quantify spatial variations in resting-state BOLD times-series, which has been applied to a number of different disease conditions including epilepsy. The ReHo method assumes that within a functional cluster, the hemodynamic characteristics of each voxel will be similar (or synchronous) to that of its neighbors,[12] and that such a similarity could be changed or modulated by different conditions.[13–15]

Low-frequency oscillation–based analysis methods have been applied extensively in epilepsy studies[13,16] and are thought to directly reflect the spontaneous activity of neurons. However, the biologic origin of low-frequency oscillations is not completely understood. Moreover, the sensitivity of low-frequency oscillation–based methods still remains uncertain.[13,17,18] Several methods have been developed to examine the amplitudes of low-frequency oscillations.[19,20] In the current study, we use the fractional amplitude of low-frequency fluctuations (fALFFs) as a measure of the strength of the low-frequency oscillations, which is the ratio of the power spectrum of low-frequency signals to that of the entire frequency range.[9,18,21]

In this study, we evaluated ReHo and fALFFs in resting-state functional MRI (fMRI) signals of subjects with a first seizure, patients diagnosed with new-onset epilepsy (NOE), and healthy controls as reference. The objective of the comparison is to determine whether these measures can differentiate subjects who had a first seizure from patients with NOE, which might eventually lead to biomarkers that are sensitive to seizure recurrence.

## Methods

### Subjects

From June 2012 until January 2015, we recruited patients from the "first-fit" service at the Maastricht University Medical Center. Patients were referred after either a neurologic outpatient consultation or a neurologic consultation at the emergency department, when it was suspected that the patient had an epileptic seizure. During the same day, patients underwent EEG, MRI, electrocardiography (ECG), and laboratory examinations, as well as additional history taking. Data were stored for research purposes. All patients had one MRI (including fMRI) scan during the study. Overall 113 patients were clinically analyzed in this period. However, only patients with a diagnosis of an "unprovoked first epileptic seizure" and "new-onset epilepsy (NOE)" were included in this study (Appendix S1). We used clinical criteria as a gold standard for the diagnosis of unprovoked first epileptic seizure and (new-onset) epilepsy using the current International League Against Epilepsy (ILAE) definitions.[2] In general, the diagnosis NOE was made after the occurrence of two or more unprovoked epileptic seizures before the inpatient clinical analysis (so-called first fit service), but some patients had EEG and/or MRI abnormalities associated with a high chance (≥60%) of seizure recurrence within the next 10 years, thus leading to the diagnosis of (new-onset) epilepsy. Special cases are those patients presenting with a first unprovoked seizure after the age of 40 years, with EEG and/or MRI abnormalities associated with epilepsy syndromes presenting during childhood, adolescence, or young adulthood. These subjects are categorized as first-seizure subjects, since the chance of recurrence was estimated as <60% in the next 10 years. We included 17 first-seizure subjects with ages between 25 and 78 years. Clinical follow-up ended on November 15th in 2015, thus follow-up ranged from 8 to 40 months. Nineteen patients with NOE were included in this study; of these NOE patients, seven patients initially had one seizure and

experienced another seizure during follow-up. The age of the NOE patients ranged between 18 and 83 years. Table 1 describes the characteristics of the included subjects with seizures (individual patient details are provided in Appendix S2).

Eighteen healthy control subjects (age range 24–48 years) were also included in this study to determine whether the fALFF was abnormal or not in the subject groups with seizures.

### Data acquisition

Resting-state fMRI data were acquired with a 3.0-Tesla unit (Achieva TX, Philips) using an echo-planar imaging (EPI) sequence with the following parameters: repetition time (TR) 2 s, echo time (TE) 35 msec, flip angle 90 degrees, 31 transverse slices, slice thickness 4 mm, pixel size 2 × 2 mm, and 195 volumes per acquisition. For anatomic reference, a three-dimensional (3D) fast-spoiled gradient echo $T_1$-weighted image set was acquired with the following parameters: TR 8.1 msec, $T^I$ 1,022 msec, TE 3.8 msec, flip angle 8 degrees, 52 sagittal slices, and 1 mm cubic voxel size.

### Image processing

Using SPM8 software,[22] the functional images were corrected for slice-timing effects and head displacements, co-registered with the anatomic template and smoothed with a kernel of 8 mm (full-width-at-half-maximum). Any signal drifts over time were corrected by removing the very low frequency components (<10 mHz). To correct for physiologic fluctuations, the time series from the cerebrospinal fluid (CSF) and white matter were included as covariates in the linear regression analysis.[23] Gray matter, white matter, and CSF voxels were segmented from the $T_1$-weighted images using the Freesurfer software application.[24] An anatomic atlas that divides the brain into 70 regions based on the gyri and sulci was calculated.[25] A number of studies using fMRI on epilepsy motivated us to define the regions of interest (ROIs), including the thalamus, hippocampus, temporal cortex, prefrontal cortex, precentral cortex, postcentral cortex, posterior cingulate cortex, inferior parietal cortex, precuneus, and occipital cortex.[9,21,26]

### Regional homogeneity (ReHo)

Kendall's coefficient of concordance (KCC) was used to measure the regional homogeneity of the time series of a given voxel with its 26 (immediate) neighbor voxels. The ReHo measure was previously proposed by Zang et al.[11] as $\mathrm{KCC} = (\sum (R_i)^2 - n(M)^2)/(1/12K^2(n^3 - n))$. KCC ranges from 0 to 1. $K$ is the number of contiguous voxels considered (in this case it is 27), $n$ is the number of ranks (data are sorted in ascending order in terms of intensity to compute the rank; in our case $n$ is the number of time points, thus 195) and $R_i$ the sum rank of the $i^{\text{th}}$ time point, and $M$ is the mean of the sum ranks and equal to $M = \frac{1}{2}(n + 1)K$.

ReHo measures the regional homogeneity (i.e., similarity in contiguous voxels) of time series and was averaged over all gray matter voxels per ROI. In other words, the method assumes that within a functional region, the hemodynamic time signature of each voxel will be similar (or synchronous) with that of its neighbors, and such a similarity could be modulated by disease conditions. Although most functional imaging analysis methods rely on a priori knowledge of the model used for the experiment, ReHo is a voxel-by-voxel, data-driven approach and does not require any prior knowledge of the experimental design.

### Fractional amplitude of low frequency fluctuations (fALFFs)

The signal time series of each voxel was (Fourier) transformed to the frequency domain, and the power spectrum was calculated in specific frequency subbands.[27,28] The fALFF is computed per voxel as the ratio of the power spectrum in a specific subband to that of the full frequency range (0–250 mHz). The ratio with the full frequency band helps to eliminate the confounding influence of the relatively strong signal of the pulsating CSF and physiologic noise.[18,29] A previous study has shown beneficial reproducibility of the fALFF measure.[18]

An increase in fALFF in patients with epilepsy was interpreted as a facilitation process such as the origination of epileptic activity and its propagation, whereas a decrease was interpreted as regional inhibition of function.[13] Most of the earlier work on low-frequency oscillations employed the commonly used frequency range of 10–80 mHz, but this range lacks specificity and blinds to the possibly relevant information of subbands.[9,30] In this study, we explore the full frequency range (10–250 mHz) and address how amplitudes of fluctuations change in specific frequency subbands. Based on recent literature,[18,31] the following frequency subbands were considered: 10–80 mHz (conventional frequency band), 10–27 mHz (slow-5), 27–73 mHz (slow-4), 73–198 mHz (slow-3), and 198–250 mHz (slow-2).[9,27,28] In a recent study it was reported that only the conventional, slow-5 and slow-3 frequency subbands contain useful information related to brain oscillations, whereas the slow-4 and slow-2 subbands mostly represent noise.[32] In the current study all the above frequency bands are evaluated with special attention to the slow-5 and slow-3 frequency bands. The technique to calculate the fALFF is fully automated and the frequency subbands are free parameters to select. To avoid overfitting of data, in this work we focused only on those frequency subbands that were previously defined in the literature (i.e., slow-3 and slow-5).[9,27,28]

### Statistical analysis

The Student's $t$-test was used for statistical assessment of any differences between first-seizure subjects and NOE patients. This was also used to determine abnormalities for NOE and first-seizure subjects relative to healthy controls.

**Table 1. Characteristics of subjects with seizures**

| | First seizure | New-onset epilepsy |
|---|---|---|
| Number | 17 | 19 |
| Male/female | 12/5 | 10/9 |
| Age in years, min-max (mean ± SD) | 25–78 (52.3 ± 13.5) | 18–83 (44.8 ± 18.6) |
| Follow-up time in months, min-max (median) | 14.0–39.6 (26.5) | 1.4–45.1 (26.4) |
| MRI abnormalities | | |
| Mesiotemporal lesions | | |
| Dysplasia | 0 | 2 |
| Gliosis/atrophy | 3 | 3 |
| Cortical gliosis[a] | 0 | 2 |
| Glioma | 0 | 1 |
| Ischemia | 1 | 1 |
| White matter lesions | 3 | 0 |
| Heterotopia | 1 | 0 |
| Epileptiform (EEG) abnormalities | 1/17 | 5/19 |
| Seizure types | | |
| Simple partial seizures | 0 | 5 |
| Complex partial seizures | 1 | 6 |
| Unspecified partial seizures | 0 | 1 |
| Generalized seizures | 16 | 15 |
| Type of epilepsy | | |
| Cryptogenic localization-related epilepsy | None | 13 |
| Symptomatic localization-related epilepsy | None | 6 |

SD, standard deviation; EEG, electroencephalography; NA, not applicable.
[a]Excluding gliosis of parahippocampal cortex.

Initial analysis was performed for the entire cerebrum to limit the number of statistical tests. Only when significant differences were found for the entire cerebrum, further (more regional) analyses were detailed to the above-mentioned ROIs. Each region was analyzed to study regional differences among the three subject groups (first-seizure subjects, NOE, and healthy controls). The possible effect of age as a confounder was tested using linear multivariate regression. Statistical significance was inferred when $p < 0.05$.

During the course of the study a number of patients who initially had one seizure developed additional seizures during follow-up. For these patients, the individual values of the BOLD measures were statistically compared to those of the healthy control group. To see whether abnormal BOLD amplitudes may represent a potential biomarker of seizure recurrence, a threshold was derived from the results of the relatively homogeneous healthy control group (reference value). This threshold served to determine which and how many individuals had abnormal BOLD values (i.e., beyond the 95% confidence intervals of the healthy control group).

# Results

### ReHo

The ReHo measures are listed in Table 2. No significant differences between the groups in any of the frequency (sub)bands were found. Therefore, in the following, only the results of the fALFF measure are outlined.

### Low-frequency oscillations

Figure 1 shows the fALFF versus frequency plot within the frequency range of 10–250 mHz for the entire cerebrum. Table 2 gives also the global fALFF in the various subbands for three subject groups.

In general, Table 2 and Figure 1 show that both the first-seizure subjects and NOE patients have lower amplitudes for the entire cerebrum in the conventional frequency range and in the lower frequency subbands (i.e., slow-4 and slow-5) than healthy controls, but higher amplitudes in the higher subband (slow-3). These amplitude differences remained significant after correction for age by linear regression. No significant effect of age could be observed in any of the groups ($p > 0.15$). Figure 2 shows group-averaged fALFF maps for first-seizure subjects and NOE patients relative to healthy controls for the slow-3 subband. The described effects remained robust when they were recalculated by excluding the (two) subjects who had EEG and/or MRI abnormalities (Appendix S2) from the first-seizure subject group.

### First-seizure subjects versus NOE

For the conventional frequency range, the fALFF of the whole cerebrum did not reveal significant differences between first-seizure subjects and NOE patients. Only for the slow-3 subband, the entire cerebrum showed a significantly ($p < 0.05$) elevated amplitude for the NOE patients in comparison to the first-seizure subjects. Brain regions for which the fALFF in NOE was significantly elevated compared with first-seizure subjects included the prefrontal cortex, precentral cortex, inferior parietal cortex, thalamus, occipital cortex, and precuneus (Table 3, Fig. 2). Figure 2C shows (spatial variation) maps of first-seizure subjects. These maps indicate the percentage of first-seizure subjects for which the fALFF in NOE is higher in comparison to first-seizure subjects.

### First-seizure subjects versus healthy controls

Although the whole cerebrum fALFF measure was higher for the first-seizure subjects than the healthy controls in each region investigated, it did not provide any significant differences in either the conventional frequency range any of the subbands. Only 6 (35%) of 17 first-seizure subjects had elevated fALFFs.

**Table 2. Regional homogeneity (ReHo) and fractional amplitude of low-frequency fluctuations (fALFFs) in first-seizure subjects and new-onset epilepsy patients for the whole cerebrum**

| | | Conventional 10–80 mHz | Slow-5 10–27 mHz | Slow-4 27–73 mHz | Slow-3 73–198 mHz | Slow-2 198–250 mHz |
|---|---|---|---|---|---|---|
| ReHo | First seizure | 15.2 ± 11.7 | 10.8 ± 13.4 | 12.7 ± 10.7 | 5.7 ± 8.8 | 6.6 ± 7.8 |
| | NOE | 5.4 ± 10.2 | 3.3 ± 11.1 | 7.1 ± 12.8 | 6.2 ± 11.0 | 0.9 ± 10.2 |
| | NOE – first seizure | −9.8 ± 15.5 | −7.5 ± 17.4 | −5.6 ± 14.5 | 0.5 ± 14.1 | −5.7 ± 12.8 |
| fALFF | First seizure | −0.3 ± 2.2 | −1.1 ± 3.9 | −0.3 ± 1.9 | 1.2 ± 1.4 | 5.5 ± 3.7 |
| | NOE | −5.2 ± 2.4[a] | −8.6 ± 4.2[a] | −4.2 ± 2.0 | 4.9 ± 1.1[a] | 9.0 ± 4.7 |
| | NOE – first seizure | −4.9 ± 3.2 | −7.5 ± 4.9 | −3.9 ± 2.8 | 3.7 ± 1.7[a] | 4.5 ± 5.9 |

ReHo, regional homogeneity; fALFF, fractional amplitude of low-frequency fluctuations; NOE, new-onset epilepsy (patient group).
[a]$p < 0.05$ (Student's *t*-test).
Numbers are percentage difference relative to healthy controls. Values: mean ± one standard error. Significant differences for first-seizure subjects and NOE patients relative to healthy controls are indicated in rows labeled "First seizure" and "NOE," respectively. Significant difference between NOE and first-seizure subjects are indicated in row labeled "NOE – first seizure."

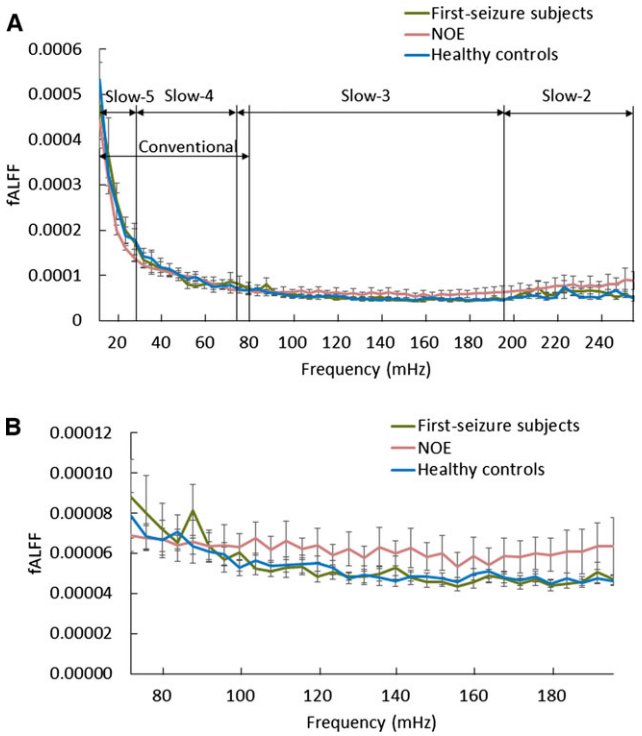


**Figure 1.**
(**A**) The fractional amplitude of low-frequency fluctuations (fALFFs) as a function of frequency for the complete frequency range of 10–250 mHz of the entire cerebrum. (**B**) Magnification of the slow-3 frequency subband. Error bars represent the standard error of the mean.


### NOE versus healthy controls

The whole cerebrum fALFF measure provided significantly lower values for NOE than healthy controls in the conventional frequency band and the slow-5 subband. The fALFF measure was significantly elevated in the slow-3 subband ($p < 0.05$). Fourteen (74%) of 19 NOE patients had elevated fALFFs.

Regional analysis for the slow-3 subband showed elevated amplitudes for the patients with NOE in all the brain regions investigated.

A stepwise increase in fALFF from healthy controls to first-seizure subjects to NOE is shown in Figure 3. Seven patients in the NOE group presented initially with one seizure and subsequently developed additional seizures during follow-up. It appeared that four of seven of these patients had significantly higher values than the reference value.

## Discussion

### Current findings

In this study, we set out to determine abnormalities in BOLD signal fluctuations of patients who recently had a first-ever seizure and patients diagnosed with NOE. Two measures were evaluated: ReHo and fALFF. These measures were explored in both the conventional resting-state frequency range and recently proposed frequency subbands in the entire cerebrum and predefined brain regions. The most notable findings were that in the slow-3 subband, NOE patients had an elevated fALFF, relative to first-seizure subjects and healthy controls; and that first-seizure subjects did not differ from healthy controls in terms of BOLD amplitudes. The ReHo measure did not show significant differences in any of the frequency subbands.

### Effect of frequency subbands

When considering the entire cerebrum averaged signal fluctuations, we found significantly elevated fALFF in the slow-3 subband for NOE patients relative to first-seizure subjects as well as healthy controls. In addition to the whole cerebrum, there were many regions for which the fALFF in NOE was significantly elevated in comparison to first-seizure subjects for the slow-3 subband, including the prefrontal cortex, precentral cortex, inferior parietal cortex, thalamus, occipital cortex, and precuneus. The group differences represent most likely an amalgamation of multiple (different) regions from different patients, which explains why the elevation of specific frequency components appears as a rather distributed cerebral effect.

In this study, NOE patients also showed significantly elevated fALFF in all the subcortical and cortical brain regions

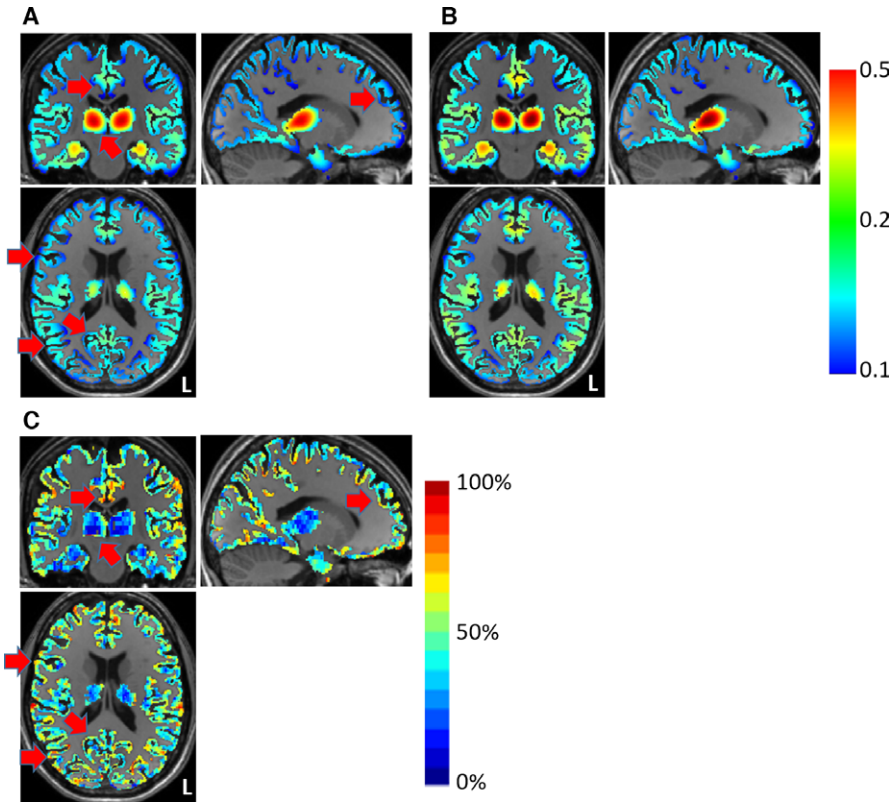


**Figure 2.**
Group-averaged functional amplitude of low-frequency fluctuation (fALFF) maps and spatial variation maps for the slow-3 subband: (**A**) group averaged fALFF map for first-seizure subjects and (**B**) new-onset epilepsy (NOE) patients, both relative to healthy controls. (**C**) Maps indicate the percentage of first-seizure subjects for which the fALFF in NOE is higher in comparison to first-seizure subjects. Brain regions where fALFF in NOE was significantly elevated compared with first-seizure subjects are the prefrontal cortex, precentral cortex, inferior parietal cortex, thalamus, occipital cortex, and precuneus. These regions are identified with the red arrows in (**A**) and (**C**).


for the slow-3 subband relative to healthy controls. This observation includes the default mode network areas, which corresponds to the effects previously reported on idiopathic generalized epilepsy using the fALFF measure.[21,33]

Our results for NOE are also in line with the findings reported in temporal lobe epilepsy using ALFF,[10] which is the not-normalized variant of fALFF. It was reported that patients show significant changes in ALFF relative to controls in various brain regions. Given that the patients developed epilepsies of various types and localizations, the widespread differences were likely caused by a mixing of different localizations, in addition to regions like the thalamus and default mode network regions, which are generically affected by many types of epilepsies.

Because the principal differences in frequency ranges between EEG and fMRI, comparison studies on the same frequency bands do not exist using the two techniques. However, in a recent study, Zhan et al.[32] showed that the EEG-derived alpha rhythm (order of 10 Hz) is correlated with the slow-3 subband of the BOLD oscillations in the visual (occipital) cortex and the attention (frontoparietal) network. In addition, in several other previous studies it has been shown that alpha waves could be abnormal in epilepsy.[34,35]

## Clinical perspectives

The current study population with the two groups (first-seizure subjects and NOE) did show some differences in terms of epilepsy type, seizure type, and MRI findings. For instance, in patients with NOE, more simple and complex partial seizures were observed in comparison to the first-seizure subjects, who had more generalized seizures. In most of the cases, patients with NOE have had two seizures, and in several cases even more than two. The (often) more subtle symptoms of a partial seizure in contrast to the more alarming symptoms of generalized seizures, make people less likely to go see a doctor after a first event, which could possibly explain these numbers. Only one first-seizure subject showed EEG abnormalities. These observations make it clear that the development of additional seizures does not depend on the prior detection of EEG abnormalities. However, in cases where EEG abnormalities are observed, the recurrence risk increases strongly, which leads to the diagnosis of NOE when following the current definition.[3] As may be expected, there is a higher number of MRI abnormalities in the NOE group. In four cases, these abnormalities are the cause of the patient's remote symptomatic epilepsy. In the first-seizure subjects, many of the MRI abnormalities are white matter lesions, which are nonspecific (presumed of vascular origin) and thus not related to the epileptic seizure. Considering the MRI and EEG findings or lack of predictive biomarkers, it would therefore be welcome when alternative measures, for instance the fALFF, might provide additional valuable information for subjects with a first seizure.

**Table 3. The fractional amplitude of low-frequency fluctuations (fALFF) in subjects with a first-seizure and patients with new-onset epilepsy in various brain regions in the slow-3 subband**

| | Slow-3 (73–198 mHz) | | |
|---|---|---|---|
| Regions of interest | First seizure | NOE | NOE – first seizure |
| Thalamus | 0.7 ± 1.6 | 4.6 ± 1.4[a] | 3.9 ± 2.1[a] |
| Hippocampus | 2.9 ± 1.3 | 5.4 ± 1.5[a] | 2.1 ± 2.0 |
| Temporal cortex | 1.7 ± 1.3 | 4.9 ± 1.3[a] | 2.8 ± 1.9 |
| Prefrontal cortex | 0.2 ± 1.4 | 4.4 ± 1.0[a] | 3.5 ± 1.7[a] |
| Precentral cortex | 0.8 ± 1.6 | 5.3 ± 1.2[a] | 3.8 ± 2.0[a] |
| Postcentral cortex | 0.6 ± 1.8 | 5.1 ± 1.4[a] | 3.5 ± 2.3 |
| Posterior cingulate cortex | 2.4 ± 1.6 | 5.9 ± 1.5[a] | 3.1 ± 2.2 |
| Inferior parietal cortex | 0.2 ± 1.4 | 6.0 ± 1.7[a] | 5.3 ± 1.8[a] |
| Precuneus | 3.1 ± 1.6 | 7.2 ± 1.2[a] | 4.1 ± 2.0[a] |
| Occipital cortex | 2.1 ± 2.0 | 7.8 ± 1.4[a] | 5.7 ± 2.4[a] |

NOE, new-onset epilepsy (patient group).
[a]$p < 0.05$ (Student's *t*-test).
Numbers are percentage difference relative to healthy controls. Significant differences between first-seizure subjects and NOE patients relative to healthy controls are indicated in rows labeled "First seizure" and "NOE," respectively. Significant difference between NOE and first-seizure subjects are indicated in the row labeled "NOE – first seizure."

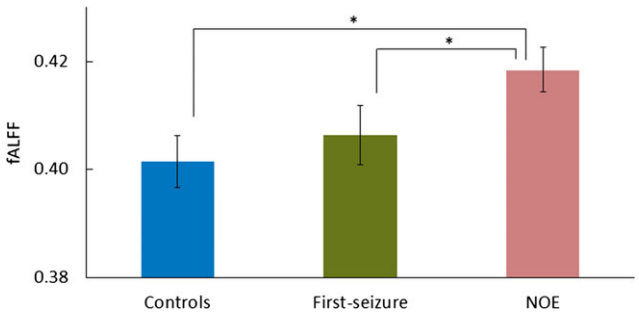


**Figure 3.**
Bar chart showing stepwise increase of the functional amplitude of low-frequency fluctuations (fALLF) in the slow-3 frequency subband going from healthy controls to first-seizure subjects and to patients with new-onset epilepsy (NOE). Error bars represent the standard error of mean. *$p < 0.05$.
*Epilepsia* © ILAE

We found that subjects with a first seizure exhibit fALFF values in the slow-3 subband that are significantly lower than for NOE. This observation could be clinically useful for identifying patients at risk for developing NOE with an unknown history of seizures. The diagnostic challenge is to differentiate between those patients who will remain seizure free and those who will experience recurrent seizures after a first seizure. In this study, seven patients who were initially diagnosed as having had one seizure, experienced recurrent seizures. We found that most of these patients had a fALFF value in the slow-3 subband that was higher than the mean fALFF of the first-seizure subjects as well as the healthy controls. This finding suggests that the fALFF can be used to determine patients who are at increased risk for developing NOE. These NOE patients did not show abnormalities on the MRI scan or EEG recordings, which shows that abnormalities might have developed after they were investigated. It could be envisioned that these patients at risk should preferably undergo additional examinations (EEG and/or fMRI) and should be monitored for elevation in fALFF during follow-up. These observations need to be investigated in more detail with larger numbers of subjects.

### Study considerations

Slow-3 subband appeared to provide the strongest differences between NOE and first-seizure subjects. It may be possible to get even larger differences between NOE and first-seizure subjects by further narrowing the frequency subband within the slow-3 range. However, this may lead to overfitting of the technique with the current dataset, and the effect would not be generalized.

For the slow-3 subband relevant effects were found, but not for the higher slow-2 subband. It has been suggested previously[36] that there could be folding of higher frequency oscillations into the lower frequency range due to aliasing. This could be a reason that no significant differences and relatively strong intersubject variations were observed in the highest (slow-2) subband. Higher fMRI sampling rates (i.e., shorter TR) may be required to see whether any characteristic NOE effects also appear for the higher frequency ranges.

In the current study, multiple quantities (subbands, brain regions) of different subject groups were statistically tested. The analyses were performed according to a hierarchical step by step approach; therefore, correction for multiple comparisons was not applied. However, to limit the number of tests, first, the effects of the fALFF in first-seizure subjects and NOE were studied primarily in the slow-5 and slow-3 subbands, whereas the healthy controls group was used only as a reference. Subsequently, when effects were seen for the whole cerebrum, regional variations were studied only for the slow-3 subband.

## Conclusions

This study is the first to report on low-frequency oscillations in patients with NOE and first-seizure subjects. As a preliminary result we found amplitude elevations of cerebral BOLD fluctuations in terms of fALFF in patients with NOE relative to subjects with a first seizure and healthy controls. In addition, abnormally high amplitudes were observed in the majority of patients who developed epilepsy during follow-up. This finding sets a new hypothesis that fALFF holds promise as a potential biomarker for identifying subjects at increased risk for developing NOE. The frequency range of 73–198 mHz (slow-3) appeared sensitive for the discrimination. As a scope for future work, a larger

study population is required to establish a threshold of fALFF with either healthy controls or NOE group as reference. The threshold will likely depend on the age of the patients, type of seizure, MRI lesions, and etiology.

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## Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Patient inclusion flow chart.

**Appendix S2.** Detailed patient characteristics.