SUPPLEMENTARY MATERIAL

METHODS

Inclusion criterium was the presence of delirium assessed by 4AT test and DSM-5 diagnostic criteria [1,2]. Exclusion criteria were mild cognitive impairment (MCI) or dementia, Parkinson’s disease, traumas, seizures, previous psychiatric disease, alcohol abuse, pre-existing EEG abnormalities, thalamic lesions (including thalamic stroke), and vast cerebrovascular lesions or brain atrophy on neuroimaging exams.

The study was performed according to the declaration of Helsinki and its later amendments, and it was approved by the local ethical committee. A written consent for research purposes was obtained from all participants.

Participants

Multicenter study

A total of 140 consecutive patients, admitted from January 2021 to December 2021, were evaluated for the incidence of delirium during hospital stay and for the presence of exclusion criteria as reported. Participants referred to the following Centers: 1. Neurology Clinic and Internal Medicine Clinic of University G. d’Annunzio of Chieti-Pescara, “SS Annunziata” Hospital of Chieti; 2. Neurology Clinic of the University of Brescia; 3. Neurology Clinic of Policlinico of Milan; 4. Neurology Clinic of Padova University; 5. Neurology Clinic of Treviso Hospital; 6. Neurology Clinic of University of Perugia; 7. Neurology Clinic of Sapienza University of Rome.

65 patients with delirium were selected and were matched for age and sex with 41 control subjects, admitted during the same period, who did not develop delirium (no-delirium group).

All patients underwent a resting state EEG (rsEEG) recording before any pharmacological treatment was initiated.
At the admission, as for study design, medical history was collected from all patients and a complete neurological examination was also performed.

Delirium subtype (hypokinetic, hyperkinetic, and mixed) and the hospital length of stay (LOS) were reported. All predisposing factors for delirium occurrence were considered, such as comorbidities, concurrent lung and urinary infections, bedding, and the presence of bladder catheter. The presence of comorbidities was assessed using the Charlson Comorbidity Index (CCI) [3], which assigns a score ranging from 1 to 6 to each disease for a total of different sixteen pathologies. The final score, which ranges up to 37, is calculated also combining the patient age.

Possible electrolyte, metabolic, hematologic, respiratory alterations were promptly corrected at admission.

Supplementary Figure 1 shows the flow chart of patient recruitment.

**Single-center longitudinal study**

The patients admitted to Center 1 wards, who developed delirium during hospital stay and included in the multicenter cohort study, were recorded with rsEEG at resolution of delirium and at 1 month after hospital discharge.

At the time of the second recording, 10 patients (those with hyperkinetic or mixed delirium) had been treated with iv Benzodiazepine (Lorazepam or Diazepam) (3 patients), im Haloperidol (5 patients) or Haloperidol + Benzodiazepines (2 patients). These treatments were promptly withdrawn after resolution of delirium. The third recording was therefore performed off treatments.

**4AT**

The 4AT [1] is a brief screening test characterized by high sensitivity and specificity in detecting the presence of delirium.

The scale is formed by four different items, which evaluate the state of alertness (first item), cognition (second and third items), and either the acute or fluctuating onset (fourth item).
The final score can range from 0 to 12 points. Delirium is diagnosed when the score is equal or superior to 4, whereas when it is between 1 and 3 a cognitive decline should be considered and better evaluated.

**Quantitative EEG recordings**

Quantitative EEG (QEEG) was recorded with Ag/AgCl disk scalp electrodes from 19 scalp derivations (Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, Pz, P3, P4, T3, T4, T5, T6, O1, and O2) placed according to the international 10–20 system, with two additional electrodes placed on A1 and A2. Recordings were acquired continuously with subjects resting comfortably, with their eyes closed. Eye movements were simultaneously monitored from two additional channels. Two pairs of bipolar recording channels for respiration and electrocardiogram were also applied. Muscular or tremor artefacts were controlled using supplementary derivations.

**EEG data analysis**

EEG recordings were obtained at each Center and centrally analyzed at Center 1. QEEG was acquired as a continuous signal for 30 minutes and visually inspected for current clinical interpretation or artefact detections. QEEG signal was stored to be epoched in off-analysis setting as series of 2-second-long epochs. Electrodes from anterior (Fp1, Fp2, Fz, F3, F4, F7, F8), posterior (P3, P4, Pz, O1, O2), and temporal (T3, T4, T5, T6) derivations were considered for the analysis. Ninety blocks of artifact-free 2-second-long epochs appearing consecutively were selected off-line by visual inspection. Fast Fourier Transform (FFT) was applied on each epoch allowing a frequency resolution of 0.5 Hz. The obtained spectra values were then processed to compute a mean power spectrum for each channel and divided automatically into four different frequency bands, as follows: delta (3–4 Hz), theta (4.5–5.5 Hz), fast theta or pre-alpha (6–7.5 Hz), and alpha (8–12 Hz).
EEG traces were quantified by the following mathematical descriptors: dominant frequency (DF), which is the frequency where the spectral power value was greatest for each epoch, and dominant frequency variability (DFV), expressing the variability of DF across the 90 analyzed epochs.

The final pattern of Compressed Spectral Array (CSA), which represents the epoch-to-epoch representation of EEG-FFT for each derivation, was then calculated. Indeed, based on mean DF (MDF), mean DFV, and frequency prevalence (FP), which expresses the percentage of epochs where dominant alpha, pre-alpha, or theta-delta frequencies were found, six different QEEG activity patterns were classified, as follows: pattern 1 (stable alpha), corresponding to the presence of a dominant alpha in 60% or more of analyzed epochs (DF $\geq$ 8 Hz, DFV $<$ 1.6 Hz); pattern 1 plus, characterized by DF in the alpha frequency band with intrinsic variability (DFV $>$ 1.5 Hz); pattern 2 (unstable alpha with pre-alpha or theta/delta), described by the presence of dominant alpha (DF $\geq$ 8 Hz, DFV $>$ 2 Hz) in $<$ 50% of epochs with dominant pre-alpha or theta (DF $<$ 8 Hz) in 40% of more of epochs; pattern 3 (stable pre-alpha), consisting of the complete absence of alpha and the presence of a stable pre-alpha (DF $<$ 8 Hz, DFV $<$ 1.0 Hz) in 70% of more of analyzed epochs; pattern 4 (unstable pre-alpha with theta/delta), consisting of absence of alpha, dominant pre-alpha in $<$ 70% of analyzed epochs, with dominant theta or delta in 40% or more of epochs (DFV $<$ 2.0 Hz); pattern 5 (unstable low frequency), characterized by the absence of both alpha and pre-alpha dominant activity in more than two subsequent epochs (DFV $<$ 4 Hz) [4,5].

**Statistical analysis**

Gaussian continuous variables were reported as mean ± standard deviation (SD), and for not gaussian as median±IRQ (interquartile range). Dichotomous and categorical variables were reported as absolute numbers and percentages. Differences between the two groups (delirium group and no-delirium group) were evaluated by analysis of variance, by quantile regression to model the effects of covariates on quantiles of a response variable, and chi-square test for continuous and categorical variables.
A post hoc power of study, effect and estimate sample size calculation were performed. In the cross-sectional study, considering the two samples (65 patients with delirium vs. 41 patients without delirium) and considering that the percentage of EEG alterations (CSA pattern 2 to 4) was 98% in the case sample and 10.9% in the control sample, the power of study is above 90%.

By using the Binomial Effect Size Display (BESD) which in our study results to be 63%, the estimate size calculation is 15 patients per group.

For the longitudinal study, we considered that 30% of patients without delirium included in the cross-sectional study showed EEG abnormalities. Therefore, we would expect that 30% of the patients, included in the longitudinal study, should still have EEG abnormalities after resolution of delirium. In our cohort only 2 (10%) patients out of the 23 included in the longitudinal study still had EEG abnormalities at follow-up. Based on these results, the power analysis showed a power of 72% and an estimated sample size of 29 patients. Further confirmation studies are needed.

All analyses were age and sex adjusted since these effects could interfere with dependent and independent variables.

All statistical analyses were performed using SAS software rel.9.4.
SUPPLEMENTARY TABLE AND FIGURES

Supplementary Table 1: Demographic, clinical and electrophysiological characteristics of delirium group considering the single Center study (Center 1).

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>83.1 (6.5)</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>Death, n (%)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Delirium Onset from the admittance *</td>
<td>2.3 (2.2)</td>
</tr>
<tr>
<td>Hyperkinetic, n (%)</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>Hypokinetic, n (%)</td>
<td>6 (26.1)</td>
</tr>
<tr>
<td>Mixed, n (%)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>LOS</td>
<td>14.9 (14.0)</td>
</tr>
</tbody>
</table>

Predisposing factors for delirium

| CCI                        | 6.6 (1.6) | 0.2 |
|----------------------------|-----------|
| Pneumonia, n (%)           | 3 (13.0)  | 0.6 |
| Urinary tract infection, n (%) | 0 (0) | 0.1 |
| Bedding, n (%)             | 15 (65.2) | 0.002 |
| Bladder catheter, n (%)    | 15 (65.2) | 0.01 |

Disease at the admittance, n (%)

| Ischemic stroke            | 13 (56.5) | 0.02 |
|----------------------------|-----------|
| Brain hemorrhage           | 5 (21.7)  | 0.5 |
| Brain tumor                | 1 (4.3)   | 0.7 |
| Other neurological diseases | 0 (0)    | 0.03 |
| Non neurological diseases  | 2 (8.6)   | 0.008 |

EEG characteristics

<table>
<thead>
<tr>
<th>Anterior</th>
<th>Temporal</th>
<th>Posterior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>DFV</td>
</tr>
<tr>
<td>During delirium</td>
<td>6.6 (1.0)</td>
<td>1.8 (1.3)</td>
</tr>
<tr>
<td>After resolution</td>
<td>8.4 (1.0)</td>
<td>0.6 (0.5)</td>
</tr>
<tr>
<td>1-month follow-up</td>
<td>8.2 (0.9)</td>
<td>0.6 (0.9)</td>
</tr>
</tbody>
</table>

All data are reported as mean (standard deviation, SD), when not differently stated.

* Delirium onset is expressed in days.
P-values refer to comparison with delirium multicenter cohort.

Abbreviations: n= number of patients; CCI= Charlson Comorbidity Index; LOS= hospital length of stay; DF= Dominant frequency; DFV= Dominant frequency variability.

Supplementary Figure 1: The flow chart of patient recruitment.
**Supplementary Figure 2:** Different CSA patterns from occipital derivations in five illustrative patients included in no-delirium group and delirium group.
**Supplementary Figure 3:** Example of a patient’s pathological EEG pattern and its recovery to normal pattern after the episode of delirium and at 1-month follow-up from three different derivations (anterior, temporal, and posterior).
LIMITATIONS OF THE STUDY

Risk factors for development of delirium

The risk of delirium increases with increasing frequency and severity of comorbidities. Comorbidities in our study was assessed by the CCI, which provides a simple and valid tool for estimating the risk of death associated with comorbidity and has proven to be reliable in different types of outcomes research [6–8].

Delirium risk factors may differ between medical and surgical patients where the latter are exposed to iatrogenic factors such as anesthetic agents or surgical procedures. In our cohort none of the patients were surgical or exposed to anesthetic agents or had fractures or other causes of neuropathic pain, which could engender TCD.

Parkinson’s disease patients were also excluded, since inhibitory projections from the basal ganglia in the pallido-thalamic tract might provoke TCD.

Patients with trauma were excluded because traumas can disrupt or modulate cortical function over spatially distributed areas through diaschisis, altering the physiological state of distant regions including the thalamocortical system [9].

Finally, all the possible metabolic, hematologic, electrolyte, respiratory imbalances were corrected in all the patients.

Most patients enrolled showed cerebrovascular disorders. This is a sensitive confounding factor as cortical lesions resulting from stroke can lead to excessive hyperpolarization of thalamic neurons, switching them to a low threshold bursting regime by de-inactivating calcium currents [10,11]. This switch may induce TCD dynamics and may entrain thalamo-cortical pathways, propagating low-frequency oscillations into the neocortex. To mitigate this confounding factor, we excluded from the study patients with thalamic and extensive hemispheric lesions [12].

As mentioned in the previous paragraph, the genesis of delirium is multifactorial [13] and generally all conditions that can induce a neuroinflammatory process are potential causes of delirium.
Through the action of inflammatory agents, a cascade of events is activated, which culminates in endothelial and microvascular damage and blood–brain barrier alterations.

Among all the delirium predisposing factors considered in the study, delirium group showed a higher CCI score (p=0.02), a higher LOS (p<0.0001), and a higher occurrence of concurrent pneumonia (p=0.02). Therefore, we cannot rule out the role of comorbidities and possible inflammatory state related to them as factors influencing the thalamocortical system function, since a link between TCD and severe sepsis has been reported [14]. Neuropathological processes following sepsis additionally lead to the development of TCD, as the patients' EEG display the distinctive slowing of the DF into pre-alpha range [14]. We could overcome this concern by demonstrating that EEG after delirium recovery normalized, even though the underlying cause of hospital stay was still active.

Methodological remarks

The TCD involvement in the pathogenesis of delirium was not corroborated by functional MRI study or a MRI study of the microstructural alterations of thalamocortical system. These studies could be helpful to clarify the involvement of the thalamus in the genesis of EEG alterations in delirium. However, this is an ecological study, performed in hospitalized patients, who, per their condition, could not undergo MRI during delirium.

A further methodological limitation lies in the small sample size of the longitudinal study, which makes the results not generalizable. The number of followed-up patients need to be increased in a further longitudinal study.

Finally, even though we did not find differences in EEG abnormalities between hypokinetic vs. hyperkinetic delirium, this might be explained by the sample size of the cross-sectional study. Increasing the number of studied patients could help proving the datum.
SUPPLEMENTARY REFERENCES


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