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# Substantia nigra hyperechogenicity and brain ventricular size as biomarkers of early dementia with Lewy bodies

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## Abstract

**Background** Diagnosis of dementia with Lewy bodies (DLB) is challenging, especially in the earlier stages of the disease, owing to the clinical overlap with other neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD). We aimed to identify the transcranial sonography (TCS) parameters that can help us to detect early DLB patients.

**Methods** In this cross-sectional study, we prospectively recruited newly diagnosed DLB patients with less than 3 years from the onset of cognitive symptoms. For comparison purposes, we also included AD and PD patients, with a disease duration of less than 3 years, and a control group. TCS was performed to assess the substantia nigra (SN) echogenicity, the width of the third ventricle, and the frontal horns of the lateral ventricles. Subsequently, TCS images were analyzed with the medical image viewer *Horos* in order to quantify the intensity of the echogenicity of the SN. Univariate analysis and a logistic regression model were used to identify which variables can predict the diagnosis of DLB.

**Results** One hundred and seven participants were included (23 DLB, 26 AD, 27 PD and 31 controls). The median age of DLB patients was 75(72–77) years, with a disease duration of 2 years. DLB and PD patients showed higher SN hyperechogenicity rates (72.73% and 81.82%, respectively) and a greater area of the SN compared to AD patients and controls ($p < 0.001$). DLB and AD patients had wider ventricular systems than the other study groups. The SN hyperechogenicity predicted a diagnosis of DLB with an odds ratio of 22.67 (95%CI 3.98; 129.12, $p < 0.001$) when compared to AD patients. Unilateral and bilateral widened frontal horns predicted diagnosis of DLB compared to PD with an odds ratio of 9.5 (95%CI 0.97; 92.83, $p = 0.053$) and 5.7 (95%CI 0.97; 33.6, $p = 0.054$), respectively.

**Conclusions** Echogenicity of the SN and widening of the frontal horns of lateral ventricles can predict the diagnosis of early DLB in this cohort of newly diagnosed patients, when compared to AD and PD patients. Transcranial sonography, a non-invasive tool, could be helpful for the diagnosis of DLB at its earlier stages.

**Keywords** Transcranial sonography, Dementia with Lewy bodies, Third ventricle width, Frontal horns of lateral ventricles, Substantia nigra

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## Background

Dementia with Lewy bodies (DLB) is the second most common cause of dementia after Alzheimer's disease (AD). The prevalence of DLB is estimated around 7.5% of all dementia cases in clinical cohorts [1, 2]. It is expected to rise in the following years due to the increase in worldwide life expectancy [2]. DLB has an important impact on the quality of life of patients and their caregivers [3, 4]. Clinically, DLB is characterized by dementia associated with visual hallucinations, fluctuations in cognition, parkinsonism and/or REM sleep behavior disorder. The diagnosis of DLB is based on clinical features and the supportive imaging and polysomnographic markers. However, the diagnosis is often challenging, mainly in the earlier stages, when a significant clinical overlap with other neurodegenerative diseases such as AD and Parkinson's disease (PD) is observed. To increase the diagnostic accuracy of DLB, it is crucial to find novel biomarkers for better management of patients affected by this disabling neurodegenerative disease.

There is not, so far, a specific biomarker for the diagnosis of DLB. Current diagnostic criteria of DLB include several clinical and imaging biomarkers, classified as indicative and supportive, depending on their diagnostic specificity [5]. Imaging biomarkers include reduced basal ganglia dopamine transporter uptake demonstrated by positron emission tomography (PET) or single-photon emission computed tomography (SPECT), a reduced uptake on $^{123}$iodine metaiodobenzylguanidine myocardial scintigraphy, or low uptake on SPECT/PET perfusion/metabolism scans with reduced occipital activity and/or the posterior cingulate island sign. However, while these imaging biomarkers, especially those assessing dopaminergic deficits, are quite specific for manifest DLB, its accuracy varies in prodromal stages [6, 7]. On the other hand, such biomarkers are invasive, expensive, and not accessible to the entire population. Therefore, the search for novel and reliable biomarkers for this disease remains of great interest. In recent years, there has been growing interest in fluid biomarkers in DLB, mostly cerebrospinal fluid (CSF) biomarkers. Many studies that examined the CSF AD biomarkers in DLB demonstrated a frequent pathological overlap between both diseases [8, 9]. Studies assessing CSF levels of synuclein, which is the pathological hallmark of DLB, and real-Time Quaking-Induced Conversion, a promising technique to detect synuclein, showed inconsistent results [10–13], and standardization of laboratory protocol methods across laboratories is needed [14, 15].

Transcranial B-mode sonography (TCS) of the midbrain structures test, is a non-invasive and easy-to-apply tool for the diagnosis of movement disorders, in particular parkinsonisms. Up to 90% of PD patients present hyperechogenicity of the substantia nigra (SN), which has been shown in the early stages and the prodromal phases of the disease[16–20]. Only a few studies have addressed the analysis of deep brain structures in clinically established DLB patients using the TCS, showing bilateral and symmetrical hyperechogenicity of the SN and larger third ventricle size when comparing PD patients with controls [21–24].

The current study aims to assess the role of TCS in the differential diagnosis of DLB at early stages.

## Methods

### Study design and patient selection

A cross-sectional study was conducted, between January 2021 and January 2023. Participants were prospectively recruited from the outpatient clinic at the Neurodegenerative diseases unit of the Hospital Universitari Germans Trias i Pujol. We included newly diagnosed patients who fulfilled the current clinical diagnostic criteria for probable DLB or probable prodromal DLB (mild cognitive impairment with Lewy bodies, MCI-LB) [5, 25], with less than 3 years from the onset of the cognitive symptoms and with a score in the Global Deterioration Scale (GDS) up to four [26]. For comparison purposes, we included a group of AD patients, following the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria for the disease [27] and a group of PD patients, who fulfilled the Movement Disorders Society (MDS) criteria for PD [28]. In both groups, the time from onset of cognitive or motor complaints, respectively, was less than 3 years, and the score on the GDS was up to four. A group of control subjects, without neurological diseases, was also recruited among the non-blood relatives of patients included in the study. This study was approved by the Ethics Committee of Hospital Universitari Germans Trias i Pujol (PI-18–114), and all participants gave their written consent to participate in the study and use their clinical data for research purposes.

### Clinical variables

Demographic and clinical data were collected from all the participants. Disease duration was defined as the time since diagnosis, but we also recorded the time from the onset of cognitive and motor complaints. Global cognition status was evaluated with the GDS, the Spanish version (MEC-35) of the Mini-Mental State Examination (MMSE) [29] and the Montreal Cognitive Assessment (MoCa) [30]. Parkinsonism severity was evaluated using part III of the MDS Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [31].

### Transcranial sonography

TCS was performed in all participants using a 2 MHz phased-array transducer (Philips Affiniti 70 ultrasound machine) by an experienced neurologist (APB) on ultrasound examination, with a standard protocol as follows: penetration depth was 14–16 cm, dynamic range 45–55 dB and moderate suppression of low echogenic signals was applied. The examination was done for both sides using the transtemporal bone window to evaluate the mesencephalic and the thalamic plane. Images were acquired in two different steps. First, a bedside analysis was performed and images were digitally stored for further off-line assessment (EchoPAC workstation, GE Healthcare) by an experienced blinded examiner (DV). The following parameters were measured: the area of the SN, the width of the third ventricle (IIIv) and the right and left frontal horns of the lateral ventricles (LV). The area of the SN was manually encircled and measured and was considered hyperechogenic if equal to or greater than 0.20 $cm^2$, according to published cut-off values [17, 33]. If one or both sides of the SN were found to be hyperechogenic, the structure was classified as such. The width of the IIIv was measured by taking the minimal transverse diameter in the thalamic plane. The IIIv normal width threshold was defined when the distance between the inner bounds of the IIIv walls was under 10 mm, according to published cut-off for people aged over 60 [32]. The right and left frontal horns of the LV were measured in the same plane and the normal width of LV frontal horn was considered when the distance between its two inner walls was under 20 mm, according to published cut-off in the same age group [32]. Secondly, digitally stored images were analyzed with the medical image viewer *Horos* by two blinded sonographers (DV and MG). *Horos* is a free and open-source code software (FOSS) program distributed free of charge under the LGPL license of Horosproject.org and sponsored by Nimble Co LLC d/b/a Purview in Annapolis, MD USA. The *Horos viewer* allowed us to measure the intensity of the echogenicity of the SN. We manually outlined the area of the SN, and the program generates a histogram of each region of interest (ROI), with the mean, minimum and maximum echogenicity (unnamed units). To analyze the morphologic changes within the SN, we performed a texture analysis, comparing the extracted variables from histograms of their echogenicity. The magnitude of the intensity of echogenicity was evaluated with the mean ROI, while the heterogeneity of this intensity was estimated with the coefficient of variation.

### Statistical analysis

Descriptive demographic, clinical and TCS data are presented as median values with interquartile ranges (25th and 75th percentiles) while the number and percentages of cases were tabulated by diagnosis. For overall group comparisons, we used Fisher's Exact test for qualitative variables and the Kruskal–Wallis test for quantitative variables. In cases where the p-value was $\leq 0.1$ in these overall comparisons, pairwise analyses were performed using Fisher's Exact test for qualitative variables or the Mann Whitney U test for quantitative variables, respectively. For the assessment of correlations, the Spearman's rank correlation coefficient was used.

To evaluate the discriminatory ability of different variables after the comparison of DLB and AD groups, as between DLB and PD patients, we calculated odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) using univariate logistic regression models. This approach aimed to identify potential independent predictive factors associated with the diagnosistic of DLB using PD or AD as the reference category, and to provide an estimation of the probability of DLB diagnosis for biomarkers with $p$-values $\leq$0,10 from the OR estimation. SPSS Version 26 (IBM Corp. Armonk, NY. USA) was used for all statistical analyses. Differences were considered statistically significant for a nominal two-sided type I error of 0.05.

## Results

### Demographic and clinical features

A total of 107 participants were included in the study: 23 DLB patients, 26 patients with AD, 27 with PD and 31 controls. Among the DLB patients, 15 fulfilled criteria of MCI-DLB and 8 of established DLB. The demographic and clinical characteristics of the participants are summarized in Table 1. The median age of DLB patients was 75 (72–77) years and 17 (73.91%) were men. The median disease duration from diagnosis was 2 (0–4) months, whereas median time from onset of cognitive complaints was 2 (1–3) years. PD patients were slightly younger compared to the DLB group (70 (64–75) years; $p=0.013$). The percentage of men was higher in the DLB and PD groups in contrast to AD and control groups ($p=0.021$ and $p=0.014$, respectively).

### Sonographic characteristics

An inadequate transtemporal bone window to assess deep brain structures was observed in 17 out of 107 (15.9%) participants. SN hyperechogenicity was observed more frequently in DLB (72.73%) and PD (81.82%) patients than in AD and controls (10.5 and 7.41%, respectively; $p<0.001$) (Table 2 and Fig. 1). The area of the SN was also significantly larger in the group with DLB and PD compared to those with AD and controls ($p<0.001$)

**Table 1** Demographic and clinical data of participants

| | DLB (*n* = 23) | AD (*n* = 26) | PD (*n* = 27) | Controls (*n* = 31) | *p*-value[A] | *p*-value DLB vs controls[B] | *p*-value DLB vs AD[B] | *p*-value DLB vs PD[B] | *p*-value AD vs controls[B] | *p*-value PD vs controls[B] | *p*-value AD vs PD[B] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age in years, median (IQR) | 75 (72–77) | 73.5 (68–76) | 70 (64–75) | 72 (63–77) | 0.079 | 0.056 | 0.119 | **0.013** | 0.471 | 0.742 | 0.270 |
| Sex male, n(%) | 17 (73.91) | 10 (38.46) | 17 (62.96) | 12 (38.71) | **0.02** | **0.014** | **0.021** | 0.546 | 1.000 | 0.113 | 0.102 |
| MDS-UPDRS III median (IQR) | 19 (13–32) | 2 (1–5.5) | 20 (14–23.5) | 0 (0–2) | **< 0.001** | **< 0.001** | **< 0.001** | 0.525 | 0.683 | **< 0.001** | **< 0.001** |
| MEC-35 median (IQR) | 30 (25.25–31.75) | 27.50 (25–30.75) | 34 (31.5–35) | 34 (32–35) | **< 0.001** | **< 0.001** | 0.374 | **< 0.001** | **< 0.001** | 0.664 | **< 0.001** |
| MoCa median (IQR)) | 18 (14.5–22) | 16 (12.5–19.75) | 25 (23–27) | 26 (24.5–28) | **< 0.001** | **< 0.001** | 0.247 | **< 0.001** | **< 0.001** | 0.177 | **< 0.001** |
| Time since diagnosis in months, median (IQR) | 2 (0–4) | 2 (0–5) | 10 (0–26) | - | **< 0.001** | - | 0.831 | **0.001** | - | - | **< 0.001** |
| Time since first motor complaint in years, median (IQR) | 2 (1–3) | - | 2 (1–4) | - | 0.389 | - | 0.819 | 0.213 | - | - | 0.435 |
| Time since first cognitive complaint in years, median (IQR) | 2 (1–3) | 3 (2–4) | 2 (1–5) | - | 0.093 | - | **0.039** | 0.135 | - | - | 0.993 |

*DLB* Dementia with Lewy bodies, *PD* Parkinson's disease, *AD* Alzheimer's disease, *IQR* interquartile range, *MDS-UPDRS III* Movement Disorders Society – Unified Parkinson's Disease Rating Scale, *MEC-35* Spanish version of the Mini-Mental State Examination, *MoCa* Montreal Cognitive Assessment

[A] Fisher's Exact test for qualitative variables or Kruskal–Wallis test for quantitative variables

[B] Fisher's Exact test for qualitative variables or Mann–Whitney U Test for quantitative variables

**Table 2** Sonographic characteristics – bedside analysis

| | DLB ($n=22$) | AD ($n=19$) | PD ($n=22$) | Controls ($n=27$) | *p*-value[A] | *p*-value DLB vs controls[B] | *p*-value DLB vs AD[B] | *p*-value DLB vs PD[B] | *p*-value AD vs controls[B] | *p*-value PD vs controls[B] | *p*-value AD vs PD[B] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Right SN area in $cm^2$, median (IQR) | 0.20 (0.14–0.25) | 0.13 (0.10–0.15) | 0.19 (0.16–0.26) | 0.11 (0.08–0.12) | **< 0.001** | **< 0.001** | **0.023** | 0.584 | **0.050** | **< 0.001** | **0.002** |
| Left SN area in $cm^2$, median (IQR) | 0.23 (0.19–0.27) | 0.11 (0.09–0.13) | 0.23 (0.16–0.3) | 0.11 (0.09–0.13) | **< 0.001** | **< 0.001** | **< 0.001** | 0.712 | 0.905 | **< 0.001** | **< 0.001** |
| SN hyperechogenicity, n(%) | 16 (72.73) | 2 (10.53) | 18 (81.82) | 2 (7,41) | **< 0.001** | **< 0.001** | **< 0.001** | 0.721 | 1.000 | **0.000** | **< 0.001** |
| Bilateral SN hyperchogenicity, n(%) | 11 (50) | 1 (5.26) | 6 (27.27) | 0 (0) | **< 0.001** | **< 0.001** | **0.002** | 0.215 | 0.413 | **0.005** | 0.099 |
| IIIv size in cm, median (IQR) | 0.68 (0.52–0.83) | 0.73 (0.58–0.92) | 0.52 (0.31–0.77) | 0.51 (0.42–0.72) | **0.017** | 0.051 | 0.367 | 0.091 | **0.009** | 0.719 | **0.015** |
| Widened IIIv, n(%) | 2 (9.09) | 5 (26.31) | 0 | 2 (7.41) | 0.058 | 1.000 | 0.412 | 0.214 | 0.217 | 0.492 | **0.017** |
| Frontal right horn of LV size in cm, median (IQR) | 1.89 (1.66–2.10) | 1.92 (1.78–2.22) | 1.77 (1.55–1.88) | 1.63 (1.49–1.91) | **0.002** | **0.004** | 0.271 | **0.028** | **0.002** | 0.420 | **0.010** |
| Widened right horn of LV, n(%) | 8 (36.36) | 7 (36.84) | 2 (9.09) | 5 (18.51) | **0.031** | 0.199 | 0.741 | **0.030** | 0.153 | 0.418 | **0.017** |
| Frontal left horn of LV size in cm, median (IQR) | 1.99 (1.85–2.07) | 1.71 (1.57–2.09) | 1.76 (1.64–1.92) | 1.76 (1.62–1.9) | **0.047** | **0.013** | 0.310 | **0.003** | 0.777 | 0.995 | 0.726 |
| Widened left horn of LV, n(%) | 9 (40.9) | 7 (36.84) | 3 (13.63) | 5 (18.51) | 0.091 | 0.192 | 1.000 | 0.082 | 0.164 | 0.700 | 0.062 |

*DLB* Dementia with Lewy bodies, *PD* Parkinson's disease, *AD* Alzheimer's disease, *SN* Substantia Nigra, *IIIv* Third ventricle, *LV* Lateral ventricle

[A] Fisher's Exact test for qualitative variables or Kruskal–Wallis test for quantitative variables

[B] Fisher's Exact test for qualitative variables or Mann–Whitney U Test for quantitative variables

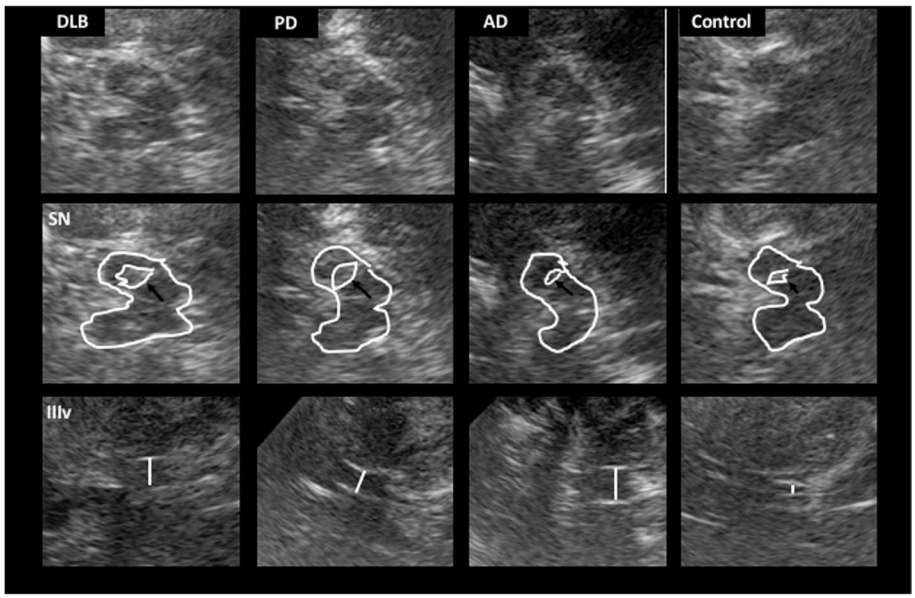


**Fig. 1** Sonographic images of mesencephalic and thalamic planes across study groups. Footnote: The figure shows ultrasound images of participants. Top row: raw images of the mesencephalic plane from patients with DLB, PD, AD and controls. Middle row: images of the mesencephalic plane, showing the perimeter of the mesencephalon (white external line) with the substantia nigra (SN) encircled (white internal line, black arrows). Bottom row: images of the thalamic plane, showing the size of the third ventricle (IIIv) (white line). The ultrasound findings include: SN hyperechogenicity and enlarged IIIv in a patient with DLB, SN hyperechogenicity with normal IIIv size in a patient with PD, normal echogenicity of the SN with enlarged IIIv in a patient with AD and normal SN echogenicity and normal IIIv size in a control subject

(Fig. 2). There were no differences regarding the area of the SN or the percentage of SN hyperechogenicity between DLB and PD patients. Eleven (50%) DLB patients had bilateral SN hyperechogenicity, in contrast to only 6 (27.27%) PD patients. Table 2 shows bedside analysis results (Table 2).

The width of the IIIv and the frontal horns of the LV were greater in the DLB and AD patients, compared to the other study groups (Fig. 2). The size of the IIIv was larger in AD patients (0.73 cm), followed by DLB patients (0.68 cm), PD patients and controls (0.52 cm and 0.51 cm, respectively). However, these differences

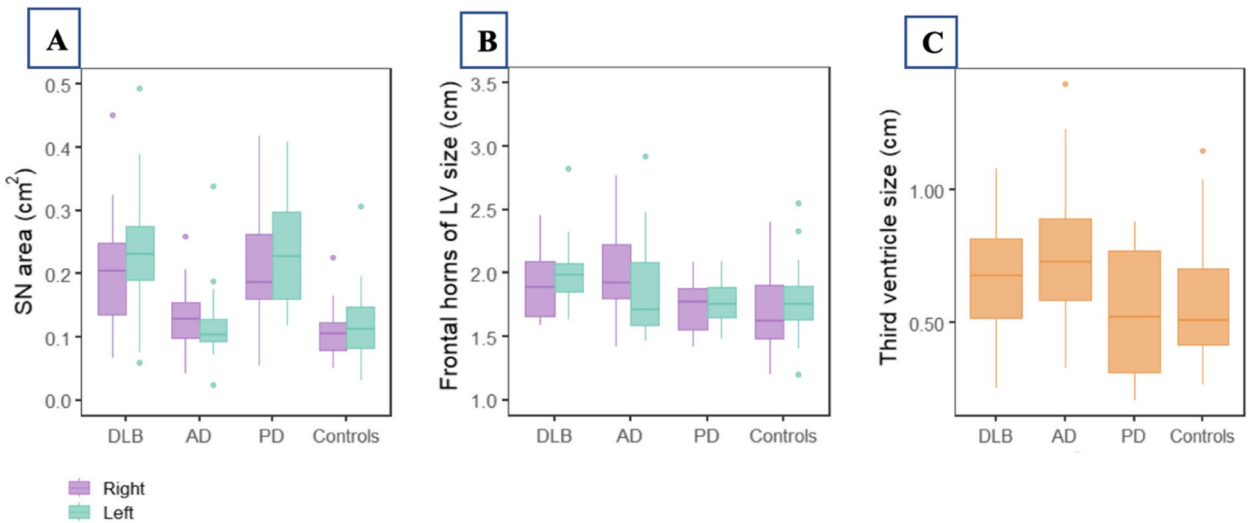


**Fig. 2** Transcranial sonography variables across study groups. **A** Comparison of right and left SN area across groups. **B** Comparison of right and left frontal horns of LV size across groups. **C** Comparison of IIIv size across groups. For each box plot, the center line, the boundaries of the box, the ends of the whiskers and points beyond the whiskers represent the median value, the interquartile range, the minimum and maximum values, and the outliers, respectively

were only significant between AD and PD patients, and between AD and controls ($p=0.015$ and $p=0.009$, respectively). Regarding the size of the frontal horns of the LV, we also observed wider horns in DLB and AD patients, with significant differences observed between DLB and controls, DLB and PD on both sides, and on the right side between AD and controls and AD and PD patients (Table 2). In addition, 11 (50%) of DLB patients and 8 (42.10%) of AD patients had a widened frontal horn of LV, compared to only 3 (13.6%) of PD patients and 6 (22%) of control patients ($p=0.017$).

We subsequently classified DLB patients into MCI-DLB and DLB to specifically assess the sonographic features in MCI-DLB patients. We did not find any significant differences in any of the sonographic parameters between MCI-DLB and DLB (Table 3). However, when comparing MCI-DLB and AD, we found that MCI-DLB patients had a larger SN area and a higher proportion of SN hyperechogenicity than AD patients (86.7% and 10.5%, respectively; p<0.001). When comparing MCI-DLB subjects and controls we observed that MCI-DLB subjects also had a larger SN area and a wider IIIv and frontal horns of LV (Table 3).

The intensity of the SN echogenicity, measured by means of the Horos viewer, was similar among all study groups. Furthermore, no differences were observed among study subjects regarding the heterogeneity of the intensity (Table 4).

A significant correlation between the severity of motor symptoms (MDS-UPDRS-III score) and the size of the right SN area was observed (correlation coefficient: 0.548; $p=0.042$) in the MCI-DLB group. No further significant correlations between motor signs and echographic features were found. The MDS-UPDRS-III score and the levodopa equivalent daily dose (LEDD) were higher in the group of DLB patients with bilateral SN hyperechogenicity compared to those with unilateral SN hyperechogenicity (20(10–43) vs 13(5–32) and 172.73 ± 211.38mg vs 120 ± 195.57mg, respectively), but these differences were not statistically significant ($p=0.307$ and $p=0.639$, respectively).

The univariate logistic regression model showed us that SN hyperechogenicity significantly predicts DLB, in comparison to AD, with an OR of 22.67 (95%CI 3.98; 129.12, $p<0.001$). In addition, both unilateral and bilateral widening of the frontal horns suggest a potential diagnostic

**Table 3** Comparison of sonographic parameters between DLB, MCI-DLB, AD and controls

| | MCI-DLB ($n=15$) | DLB ($n=8$) | AD ($n=19$) | Controls ($n=27$) | *p*-value MCI-DLB vs DLB | *p*-value MCI-DLB vs AD | *p*-value MCI-DLB vs controls |
|---|---|---|---|---|---|---|---|
| Right SN area in $cm^2$, median (IQR) | 0.20 (0.12–0,25) | 0.20 (0.12–0.25) | 0.13 (0.10–0.15) | 0.11 (0.08–0.12) | 0.970 | **0.044** | **0.001** |
| Left SN area in $cm^2$, median (IQR) | 0.23 (0.20–0.26) | 0.23 (0.11–0.32) | 0.11 (0.09–0.13) | 0.11 (0.09–0.13) | 1 | **<0.001** | **<0.001** |
| SN hyperechogenicity, n | 13 (86.7%) | 4 (50%) | 2 (10.5%) | 2 (7.4%) | 0.056 | **<0.001** | **<0.001** |
| Bilateral SN hyperchogenicity, n | 8 (53.3%) | 3 (37.5%) | 1 (5.3%) | 0 (0%) | 0.769 | **0.001** | **<0.001** |
| IIIv size in cm, median (IQR) | 0.73 (0.52–0.89) | 0.60 (0.44–0.75) | 0.73 (0.58–0.92) | 0.51 (0.42–0.72) | 0.259 | 0.636 | **0.027** |
| Frontal right horn of LV size in cm, median (IQR) | 1.97 (1.68–2.13) | 1.77 (1.64–1.94) | 1.92 (1.78–2.22) | 1.63 (1.49–1.91) | 0.173 | 0.591 | **0.004** |
| Frontal left horn of LV size in cm, median (IQR) | 2.01 (1.91–2.17) | 1.90 (1.66–2.00) | 1.71 (1.57–2.09) | 1.76 (1.62–1.9) | 0.099 | 0.158 | **0.006** |

*MCI* Mild Cognitive Impairment, *DLB* Dementia with Lewy bodies, *AD* Alzheimer's disease, *SN* Substantia Nigra, *IIIv* Third ventricle, *LV* Lateral ventricle, *IQR* Interquartilic range

**Table 4** Sonographic characteristics – Horos analysis

| | DLB ($n=23$) | AD ($n=26$) | PD ($n=27$) | Controls ($n=31$) | *p*-valueA |
|---|---|---|---|---|---|
| Intensity of the right SN echogenicity | 61.30 (46.39–80.50) | 65.87 (50.91–102.10) | 67.89 (54.92–96.58) | 83.38 (52.60–96.80) | 0.358 |
| Heterogeneity of the right SN echogenicity (CV) | 30.90 (25.00–37.80) | 28.26 (22.26–31.03) | 25.29 (20.74–27.92) | 23.72 (20.53–34-54) | 0.237 |
| Intensity of the left SN echogenicity | 62.20 (40.13–78-48) | 72.14 (48.83–95.46) | 68.91 (56.23–85.64) | 68.97 (51.74–89.53) | 0.369 |
| Heterogeneity of the left SN echogenicity (CV) | 31.46 (22.38–36.39) | 26.17 (23.50–35.32) | 23.46 (22.76–31.48) | 25.39 (22.10–29.25) | 0.414 |

Data represent median and IQR (25th and 75th percentiles) (unnamed units). *CV* Coefficient of variation

[A] Kruskall-Wallis test without pairwise comparisons due to the absence of overall statistical significance with a two-sided $p \leq 0.1$

association for DLB when compared to PD, with ORs of 9.5 (95%CI 0.97; 92.83, $p=0.053$) and 5.7 (95%CI 0.97; 33.6, $p=0.054$), respectively, even though these results are not statistically significant (Table 5). Finally, using the logistic regression model, we estimated that in patients with unilateral or bilateral widened frontal horns of LV, the probability of having DLB, in comparison with PD, was 83% and 75%, respectively. Similarly, if the patient has SN hyperechogenicity, the probability of diagnosing DLB, compared to AD, is 88% (Table 6).

## Discussion

In the current study, we assessed whether TCS can be a useful tool for the differential diagnosis of DLB at earlier stages. The main findings were that SN hyperechogenicity predicts the diagnosis of DLB, when compared to AD (OR 22.67). Also, in this cohort of patients we found that bilateral hyperechogenic SN was nearly twice as frequent in DLB compared to PD patients (50% vs 27.2%, respectively).

The etiological diagnosis of cognitive decline at its earliest stages is challenging. The clinical differences between DLB and AD, the most common neurodegenerative diseases responsible for cognitive decline, could be scarce at the beginning of the memory complaints and misdiagnosis are common especially in cases with AD co-pathology [33]. In addition, the increasing scientific interest in earlier detection of these diseases, since the emergence of new therapies for AD such as monoclonal antibodies, makes mandatory to find better early diagnostic biomarkers. At this point, it appears that CSF markers are the most accurate in discriminating between patients with DLB and AD in the MCI stage [34]. However, further studies, particularly with a prospective design are needed to assess their clinical usefulness in DLB, considering the important pathological overlap among both diseases. In addition, the lumbar puncture is an invasive procedure, and not all patients can undergo or are willing to accept this technique.

TCS is a safe, easy-to-apply, cheap and non-invasive procedure, used regularly to assess patients with movement disorders, such as PD. The role of TCS in the diagnostic work-up of patients with dementia has not been thoroughly explored. Few previous studies have examined SN echogenicity in DLB patients [21–23], where bilateral hyperechogenicity of SN was consistently observed. However, most of the patients included in these studies had a disease duration longer than 2 years and, importantly, the diagnosis of these patients was made based on the previous Consensus research criteria for DLB (2005) [35], which are now considered to be potentially less sensitive and specific than the current ones [36]. Our cohort of patients, in the earliest stages of DLB, with a median duration of just 2 months since diagnosis, including patients with MCI-DLB, support that our findings refer to early stages of DLB and, therefore, could be used as possible prodromal biomarkers of the disease, if replicated in future studies. In line with previous reports, we found a high percentage of unilateral (72%) and bilateral SN hyperechogenicity

**Table 5** Univariate logistic regression analysis for differences between DLB vs PD and DLB vs AD disease

| Comparison | Factor | OR (95% CI) | *p*-value |
|---|---|---|---|
| DLB vs AD | SN hyperechogenicity | 22.67 (3.98–129.12) | **< 0.001** |
| | Widened IIIv | 0.34 (0.06–1-98) | 0.230 |
| | Unilateral widened frontal horn of LV | 2 (0.3–13.17) | 0.471 |
| | Bilateral widened frontal horns of LV | 0.8 (0.18–3.46) | 0.765 |
| DLB vs PD | SN hyperechogenicity | 0.59 (0.14; 2.48) | 0.474 |
| | Widened IIIv | Not applicable* | 0,214 |
| | Unilateral widened frontal horn of LV | 9.5 (0.97; 92.83) | 0.053 |
| | Bilateral widened frontal horns of LV | 5.7 (0.97; 33.60) | 0.054 |

* OR not estimable due presence of 0 patients with widened IIIv with PD. In this cases *p*-value was calculated by means Fisher's Exact test

**Table 6** Estimated probability of diagnosis

| Reference | Estimation on | SN hyperechogenicity | | Widened frontal horn of LV | | |
|---|---|---|---|---|---|---|
| | | No | Yes | No | Unilateral | Bilateral |
| PD | DLB | 0.6 | 0.47 | 0.34 | 0.83 | 0.75 |
| AD | DLB | 0.26 | 0.88 | 0.55 | 0.71 | 0.5 |

Estimated probability using presence of SN hyperchogenicity or widened frontal horn of LV as independent diagnostic factors

among DLB patients (50%). However, this percentage are lower than those reported previously (87–100% for unilateral SN hyperechogenicity, 40–80% for bilateral [21–23]). This could be explained, at least partly, by the shorter disease duration of our cohort although previous studies in PD had shown that SN hyperechogenicity is not a marker of disease severity or duration [37, 38]. Longitudinal studies are needed in DLB patients to replicate our findings.

The explanation for the SN hyperechogenicity is still under discussion. Several imaging investigations, experimental studies in animal models and post-morten analyses in humans, support the hypothesis that alterations in local iron deposition and changes in the cellular composition of the SN lead to its hyperechogenicity [18, 39, 40]. The evaluation of SN composition in post-mortem DLB specimens could be of great interest to deep into this important aspect.

We also found that widened unilateral or bilateral frontal horns of the LV predict diagnosis of DLB, when compared to PD. The measurement of the IIIv size and the frontal horns of the LV with TCS has been observed that closely match that observed in magnetic resonance imaging and computed tomography studies [41, 42]. Thus, our results could reflect the brain atrophy observed earlier in DLB and AD than in PD. In fact, IIIv width in TCS has been proposed as a surrogate marker of brain atrophy and a promising marker of preclinical brain atrophy [41]. As we previously observed in a population-based study, IIIv width assessed by TCS was an independent predictor of long-term cognitive impairment [43]. Only two previous small studies have measured the IIIv and frontal horns of LV by TCS in DLB patients [21, 22]. In both, larger widths of IIIv in DLB patients were observed, in comparison with PD and controls. However, patients had a longer disease duration, ranging from 2.6 to 3.7 years. Similarly to previous studies, we found that the IIIv width was greater in DLB and AD patients compared to PD and controls. Nevertheless, these differences were only statistically significant between AD and PD patients, and between AD and controls. We also observed that the size of the frontal horns of LV was larger in the DLB and AD, compared to PD patients and controls. Our findings suggest that the size of the IIIv and the LV could be surrogate markers of brain atrophy in DLB and, therefore, could be used as part of the diagnostic work-up of DLB patients.

According to our findings, the medical image Horos *viewer* seems not to be useful in quantifying the intensity of the echogenicity of the SN in these patients. This could be due to our limited sample size, but more studies are needed to corroborate these findings.

Our study has several limitations. First, an inadequate transtemporal bone window was observed in 15.9% of participants. Although this is an intrinsic limitation of the technique, similar data were reported in European population [44]. Second, the small sample size may result in reduced statistical power to detect significant differences in some ultrasound variables, specially the IIIv enlargement. Additionally, due to the small sample size, we conducted unadjusted analyses. We acknowledge that future validation in larger, multicenter studies will be crucial. The wide confidence intervals observed for some variables, particularly the OR for SN hyperechogenicity, also suggest a degree of uncertainty in these associations. Third, we did not match study groups based on gender or age. Finally, the lack of neuropathological confirmation of the diagnosis makes possible a misdiagnosis in some patients.

## Conclusion

Our findings indicate that TCS may be a useful tool for neurologists when approaching patients with cognitive decline, especially when DLB is suspected. The presence of SN hyperechogenicity supports the diagnosis of DLB rather than AD, while the widened frontal horns of the LV make the diagnosis of DLB more plausible than PD. Although prospective studies are needed, these results support the use of TCS in the diagnostic work-up of cognitive decline in routine clinical practice.

### Abbreviations

| | |
|---|---|
| DLB | Dementia with Lewy bodies |
| AD | Alzheimer's disease |
| PD | Parkinson's disease |
| PET | Positron emission tomography |
| SPECT | Single-photon emission computed tomography |
| CSF | Cerebrospinal fluid |
| TCS | Transcranial B-mode sonography |
| SN | Substantia nigra |
| GDS | Global Deterioration Scale |
| MDS | Movement Disorders Society |
| NIA-AA | National Institute on Aging and Alzheimer's Association |
| MDS-UPDRS | MDS Unified Parkinson's Disease Rating Scale |
| MMSE | Mini-Mental State Examination |
| MoCa | Montreal Cognitive Assessment |
| LV | Lateral ventricles |
| FOSS | Free and open-source code software |
| ROI | Region of interest |
| SD | Standard deviation |
| OR | Odds ratio |

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### Authors' contributions

APB conceptualized and designed the study, carried out data analyses and drafted the manuscript and figures. JR contributed to the statistical analysis and drafting of the manuscript. MG contributed to recruiting participants, in the acquisition of data, carrying out data analyses and drafting the figures. NRL contributed to the data analyses. LI and LG contributed to recruiting participants, the study design and the drafting of the manuscript. CC and SM contributed to the study design and acquisition of data. RA and PP contributed to recruiting participants. PP and KB contributed to the drafting

of the manuscript. DV contributed to the study design, recruiting participants, carried out data analyses and drafting of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate**
The study was approved by the Ethics Committee of Hospital Universitari Germans Trias i Pujol (PI-18–114). All participants provided informed consent by the Declaration of Helsinki and local clinical research regulations.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's Note