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Respiratory dysrhythmia in Dementia with Lewy bodies: a cross-sectional study

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Key words: ataxic breathing, dementia with Lewy bodies, Alzheimer's disease, polysomnographic recordings, Fast Fourier Transform

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Article Focus

In this study, we first indicated the presence of dysrhythmic breathing in the patients with Dementia with Lewy bodies, compared to the patients with Alzheimer's disease and the patients without dementia.

Key messages

The dysrhythmia of the breath-to-breath time on bed rest with closed eyes is significantly higher in the patients with Dementia with Lewy bodies.

The recording of the breath-to-breath time on bed rest with closed eyes can be a useful marker for the diagnosis of Dementia with Lewy bodies.

Strengths and Limitations

Dysrhythmic breathing is a completely novel topic in Dementia with Lewy bodies.

This study is a cross-sectional, small-sized pilot study.

The patholocgical diagnosis of Dementia with Lewy bodies could not be obtained.

ABSTRACT

Objectives: Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD). DLB is characterized by intracytoplasmic inclusions called Lewy bodies that are often seen in the brainstem. Because modulation of the respiratory rhythm is one of the most important functions of the brainstem, DLB patients may exhibit dysrhythmic breathing. This hypothesis has not yet been systematically studied. Therefore, we evaluated the association between DLB and dysrhythmic breathing.

Design: This was a cross-sectional study where consecutive inpatients who were admitted for the evaluation of progressive cognitive impairment were enrolled. We assessed breathing irregularity using polysomnographic recordings on bed rest with closed eyes, without reference to the clinical differentiation among DLB, AD and having no dementia.

Setting: Single center in Japan.

Participants: Fourteen DLB patients, twenty-one AD patients and twelve non-demented patients were enrolled in this study.

Primary outcome measures: The coefficient of variation (CV) of the breath-to-breath time was calculated. We also examined the amplitude spectrum A(f) obtained using the Fast Fourier Transform and Shannon Entropy S of A(f) in DLB patients compared to AD patients

and non-demented patients.

Results: The values of CV and Entropy S were significantly higher in the DLB patients than in the AD and non-demented patients. No significant differences were found between the AD patients and the non-demented patients.

Conclusions: DLB patients exhibit dysrhythmic breathing compared to AD and non-demented patients. Dysrhythmic breathing is a new clinical feature of DLB and the spectral analysis of breathing patterns can be clinically useful for the diagnostic differentiation of DLB from AD.

1. INTRODUCTION

Dementia with Lewy bodies (DLB) is a neurodegenerative disease characterized by parkinsonism, visual hallucinations and cognitive fluctuations. DLB is now thought to be the second most common form of dementia after Alzheimer's disease (AD), affecting 15-25% of elderly demented patients[1]. The clinical diagnostic criteria for DLB were first published in 1996 and modified in 2005[1,2]. The central feature of DLB is progressive cognitive decline. The core features include recurrent visual hallucinations, spontaneous features of parkinsonism and fluctuating cognition with pronounced variations in attention and alertness. These diagnostic criteria require clinical evaluation by a trained neurologist and include few objective markers. Although Single Photon Emission Computed Tomography (SPECT) and ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy are useful for making the differential diagnosis of DLB[3-5], these examinations are too expensive to be generally utilized. DLB is characterized by intracytoplasmic inclusions called Lewy bodies that consist of filamentous protein granules composed of alpha-synuclein and ubiquitin. Lewy bodies are often seen in the brainstem and in limbic and cortical neurons[2]. On the other hand, the brainstem serves as the connection among the cerebral hemispheres and the cerebellum, and is responsible for basic vital functions. Modulation of the respiratory rhythm is one of the most important functions of the brainstem. In cases of brain disorders, such as Wallenberg

syndrome and brain tumors, it is known that respiratory patterns sometimes become ataxic. Because brainstem neurodegeneration is often seen in patients with DLB, the respiratory patterns of DLB patients might be dysrhythmic. However, this hypothesis has not yet been systematically studied and no controlled data have been published to date. The present investigation was performed in patients with DLB, patients with AD and non-demented patients to assess and compare breathing patterns. In addition, we evaluated the usefulness of the measurement of breathing patterns as a novel tool to aid the differential diagnosis of dementia.

2. METHODS

2.1. Subjects

The study population comprised consecutive inpatients of the Department of Geriatric Medicine at the University of Tokyo Hospital, who were admitted for the evaluation of progressive cognitive impairment. The patients underwent neuropsychological assessments, including the Mini-Mental State Examination (MMSE), blood tests, and neuroimaging tests (Magnetic Resonance Imaging (MRI) and SPECT). The diagnosis was made at a consensus conference of physicians and neurologists. The diagnosis of DLB was based on the clinical diagnostic criteria proposed by McKeith et al in 2005[2]. And AD was diagnosed

in accordance with the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [6]. The non-demented group comprised the patients who did not fit the criteria for dementia in the medical and neurological examinations. Between November 2010 and June 2012, 70 patients were enrolled in this study.

The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

2.2. Exclusion Criteria

We evaluated the breathing patterns of DLB patients, AD patients and non-demented patients. Patients with cognitive impairments other than AD or DLB (e.g. normal pressure hydrocephalus, vascular dementia) were excluded.

Breathing irregularities are associated with certain environments such as high altitudes, medical conditions such as heart failure and chronic obstructive pulmonary disease, and the usage of opioids or levodopa[7,8]. We excluded one patient who reported breathing problems, including dyspnea. We also excluded four patients who were taking levodopa and dopamine-agonists. No patients were using opioids. We excluded three patients whose recorded respiratory signal data were insufficient due to noise.

2.3. Recordings of respiration

The patients underwent thirty minutes or more of recordings of respiration on bed rest with closed eyes in the inpatient ward by using the device for polysomnography (Somnotrac Pro, CareFusion, San Diego, CA, USA). The recordings included two electroencephalogram (EEG) leads (C3-A2 and O2-A1), electrooculogram and submental electromyogram (EMG). Oronasal thermistor channel and arterial oxygen saturation (finger oximetry) were also monitored. All recordings were scored visually by an experienced rater according to the standard criteria[9].

Five consecutive minutes of stable respiratory signals measured while the patients were awake were extracted from the recordings. Stable respiratory signals during wakefulness were identified using the respiratory signals themselves, arterial oxygen saturation, EMG and EEG. Wakefulness was confirmed using EEG. When the amplitude of the EMG signal that detected any body movements was high, that part of the signal was considered to have occurred during movement and was determined to be inappropriate for analysis. Epochs including apneas and hypopneas were also excluded.

2.4. Analysis of Respiratory Signals

Five minutes of stable respiratory signals were analyzed. The breath-to-breath time was calculated for each respiration. To assess breathing irregularities, the coefficient of variation

([standard deviation/mean] × 100) for the breath-to-breath time was calculated. The respiratory rate was also calculated.

Additionally, we examined the amplitude spectrum A(f) obtained using Fast Fourier Transform (FFT) for analyzing oscillation patterns in the respiratory signals. A(f) represents the amplitude distribution as a function of frequency. To avoid the possibility of spectral leakage, the signals were windowed by multiplying them by a Hamming window (w[n]):

$$w[n] = 0.54 - 0.46 \cos (2\pi n/N) \text{ for } n = 0,1,2,\cdots,N - 1$$

Then, the amplitude spectrum of the respiratory signals was analyzed using the FFT of the Hamming-windowed signal[10]. Furthermore, according to Shannon Entropy, we determined the spectral entropy S based on normalized A(f) in order to assess breathing irregularities:

$$\text{entropy } S = - \sum A(f) \cdot \log_2 (A(f))$$

To reduce the influence of artifact in the respiratory signals and FFT, we restricted the frequency of analyzing Shannon Entropy. Based on the results of the breath-to-breath time analysis (1.7 to 7.6 seconds, namely 0.13 to 0.59 Hz), we determined the validated frequency of 0.1 Hz to 0.6 Hz.

2.5. Statistical Analysis

The distribution of data was examined using the Shapiro-Wilk test. If data were normally

distributed, one way analysis of variance with Games-Howell post-hoc tests were applied for group comparisons. If the data deviated significantly from normality, the Kruskal-Wallis test was used, followed by evaluation with the Mann-Whitney U test for multiple comparisons, with the p values being corrected according to the Bonferroni method. In correlation analysis, the Spearman rank correlation coefficient was used. The $\chi 2$ test was used to compare categorical variables, such as gender.

The diagnostic cutoff points for the coefficient of variation value and Shannon Entropy S to discriminate between DLB and AD were estimated for each outcome by maximizing the Youden index. The discrimination ability was assessed by the area under the curve (AUC). Using this threshold, the sensitivity and specificity were calculated.

All of the statistical analyses were performed using the SPSS software program (version 19.0, SPSS inc., Chicago, IL, USA). Statistical significance was defined as P values < 0.05.

3. RESULTS

3.1. Patient Characteristics

Fourteen patients with DLB, twenty-one patients with AD and twelve non-demented patients were enrolled in this study. Among the fourteen patients in the DLB group, nine

patients had probable DLB and five patients had possible DLB. The diagnoses in the five possible DLB patients were all supported by the typical findings in SPECT: generalized low uptake, reduced occipital activity, and relatively preserved hippocampal blood flow. Table 1 shows the characteristics of the subjects. The age and sex distributions were not significantly different among the three groups. No significant difference was found between the DLB group and the AD group in the MMSE. The use of medications for hypertension, hyperlipidemia and diabetes mellitus were similar between the groups. Four patients in the DLB group, five patients in the AD group and no patients in the non-demented group had taken donepezil.

Table 1 - Characteristics of DLB patients, AD patients and non-demented patients.

Characteristics	DLB patients	AD patients	Non-demented	p Value
Number of subjects	n = 14	n = 21	n = 12	
Age (years)	81.5 (5.6)	79.6 (7.8)	78.5 (4.3)	n.s.
Sex (men / women)	6 / 8	7 / 14	4 / 8	n.s.
MMSE	21.0 (3.8)	21.2 (3.4)	27.8 (2.1)	< 0.001*
Hypertension	4	9	3	n.s.
Hyperlipidemia	2	1	0	n.s.
Diabetes mellitus	1	1	1	n.s.

Values expressed as mean (standard deviation) or number. * = one way analysis of variance with Games-Howell post-hoc tests (DLB vs AD : n.s., DLB vs Non-demented : $p < 0.001$, AD vs Non-demented : $p < 0.001$); AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; MMSE = Mini-mental State Examination; n.s. = not significant.

3.2. Breathing Patterns

Figure 1 shows examples of flow signals during wakefulness for a DLB patient, an AD patient and a non-demented patient. Figure 2 shows examples of the characteristic patterns of the amplitude spectrum A(f). The AD patient and the non-demented patients exhibited a sharp peak in the spectrum. On the other hand, the amplitude spectrum of the DLB patient was distributed over the whole displayed frequency area. These tracings indicate the occurrence of more irregular breathing patterns in the DLB patient compared with that observed in the AD patient and the non-demented patients.

The respiratory rates calculated from the average breath-to-breath time in the DLB patients, the AD patients and the non-demented patients were 16.2 (3.2), 17.7 (2.7) and 18.0 (2.3) per min, respectively (mean (standard deviation)). These differences were not statistically significant. On the other hand, the coefficient of variation (CV) value for the breath-to-breath time in the DLB patients was significantly higher than that in either the AD patients or the non-demented patients (13.5 (2.6), 10.0 (3.0) and 9.9 (2.8), respectively) (Figure 3A). To discriminate the DLB patients from the AD patients using the CV value, the most favorable diagnostic threshold was found to be 10.2 (AUC = 0.79). This threshold had a sensitivity of 92.9% and a specificity of 61.9%.

The results of the comparison of Shannon Entropy S are summarized in Figure 3B. The

values of Shannon Entropy S were significantly higher in the DLB patients than in the AD patients and the non-demented patients (6.35 (0.11), 6.11 (0.29) and 6.16 (0.19), respectively). To discriminate the DLB patients from the AD patients using the Shannon Entropy S value, the most favorable diagnostic threshold was found to be 6.18 (AUC = 0.77). This threshold had a sensitivity of 100% and a specificity of 57.1%.

These findings indicate the diversity of breathing frequencies, that is, respiratory dysrhythmia, in DLB patients.

3.3. Comparison of CV and Shannon Entropy S

In order to assess breathing irregularities, we used two different methods, namely, we compare CV and Shannon Entropy S. These two methods are independent approaches to the assessment of breathing patterns; however, a significant correlation (Spearman $r = 0.78$, $p < 0.001$) was observed between these two values (Figure 4).

4. DISCUSSION

In this study, we observed that patients with DLB exhibit dysrhythmic breathing compared to patients with AD and non-demented patients.

The modulation of the respiratory rhythm is closely associated with the brainstem. In particular, the pre-Bötzinger complex (pre-BötC) and the retro-trapezoid nucleus/parafacial

respiratory group (RTN/pFRG) are thought to be very important for respiratory rhythm regulation[11-13]. For this reason, respiratory dysrhythmia may occur in cases of brainstem disorders, such as Wallenberg syndrome and brain tumors. In DLB patients, Lewy bodies are often seen in the brainstem; however, it remains unknown whether the localization and density of Lewy bodies are strongly associated with the symptoms of DLB. It is possible, considering the neurodegenerative aspects of DLB, that localization of Lewy bodies in the brainstem causes respiratory dysrhythmia. One report has indicated that visual hallucinations are associated with increased numbers of Lewy bodies in the temporal lobe and amygdala, each of these areas being implicated in the generation of complex visual images[14]. In addition, concerning the association between respiration and DLB, Mizukami et al. reported the occurrence of decreased ventilatory responses to hypercapnia in DLB patients[15]. Furthermore, respiratory insufficiency, sleep-disordered breathing and central respiratory failure are known to occur in patients with multiple system atrophy[16,17], which is an alpha-synucleinopathies, similar to DLB.

In this study, we also analyzed the breathing patterns of non-demented patients. The coefficient of variation for breath-to-breath time in the non-demented patients was not significantly different from that reported in previous studies of control subjects[18,19]. DLB patients exhibit many clinical features other than dementia, visual hallucinations and

parkinsonism. For example, Rapid Eye Movement sleep behavior disorder, severe autonomic dysfunctions such as orthostatic hypotension, repeated syncope and systematized delusions can be seen in DLB patients. Furthermore, in a previous study, we reported a high frequency of periodic limb movements in DLB patients[20]. The results of the present study indicating that DLB patients exhibit dysrhythmic breathing compared to normal subjects suggest that irregular breathing patterns may be a new clinical feature of DLB.

Currently, DLB and AD are diagnosed according to their respective clinical diagnostic criteria[2,6], and differentiation of these two diseases is frequently difficult. Our findings of different breathing patterns between DLB and AD patients suggest the usefulness of the spectral analysis of breathing for discriminating patients with DLB from those with AD. Because the diagnostic threshold had a high sensitivity in our study, the spectral analysis of breathing may be useful for making an exclusive diagnosis. While the utilization of SPECT and MIBG myocardial scintigraphy are limited to well-equipped hospitals, the spectral analysis of breathing can be performed more easily and with lower expenses. As a screening tool for the diagnosis of DLB, the spectral analysis of breathing patterns may be cost-effective and useful.

The FFT is an important tool for digital signal processing of the information commonly

encoded in the sinusoids that form the signal. Additionally, the important information to be evaluated is the frequency and amplitude of the component sinusoids. To reduce spectral noise, a Hamming window is used that involves the multiplication of the signal by a smooth curve. The result is plotted graphically in terms of amplitude and frequency. In addition, we used Shannon Entropy in this study to quantify the variability of the amplitude spectrum, namely breathing irregularities. This measure has been widely used in a range of biological applications where quantitative descriptions of data regularity are required[21,22]. The Shannon Entropy indicates the degree of uncertainty and is higher when the variability of the parameter is greater.

There are several limitations to the present study. First, we included patients with possible DLB and probable DLB in the same DLB group. Additionally, we did not make a pathological diagnosis of DLB or AD. A prospective investigation on the course of breathing patterns and cognitive impairment, including the eventual pathological diagnosis, should be examined in a future study. Second, no arterial blood gas analyses were performed. Therefore, a possible effect of hypercapnia or hypocapnia on breathing cannot be excluded. To evaluate more precisely, arterial blood gas analyses should be examined in a future study, as well. Third, the number of patients in each group was relatively small. However, our data provide the first evidence of irregular breathing in DLB patients. In a future study, an

additional investigation involving a larger number of subjects should be performed.

In conclusion, we found that DLB patients exhibit dysrhythmic breathing compared to that observed in AD patients and non-demented patients. Ataxic breathing may be a new clinical feature of DLB, and the spectral analysis of breathing patterns may be clinically useful for the diagnostic differentiation of DLB from AD.

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Declaration of Interest: This was not an industry supported study. The authors have indicated no financial conflicts of interest.

Authorship responsibility: Shinichiro Hibi was involved in design, analysis, interpretation, and drafting of article. Yasuhiro Yamaguchi was responsible for conception, design, analysis, interpretation, and drafting of article. Yumi Umeda-Kameyama and Katsuya Iijima were

involved in design. Miwako Takahashi and Toshimitsu Momose were involved in analysis. Masahiro Akishita and Yasuyoshi Ouchi were involved in design and interpretation. All authors had full access to the data and take responsibility for its integrity and the accuracy of the analysis.

Ethics of investigation: The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

Data sharing: All authors had full access to the data and take responsibility for its integrity and the accuracy of the analysis.

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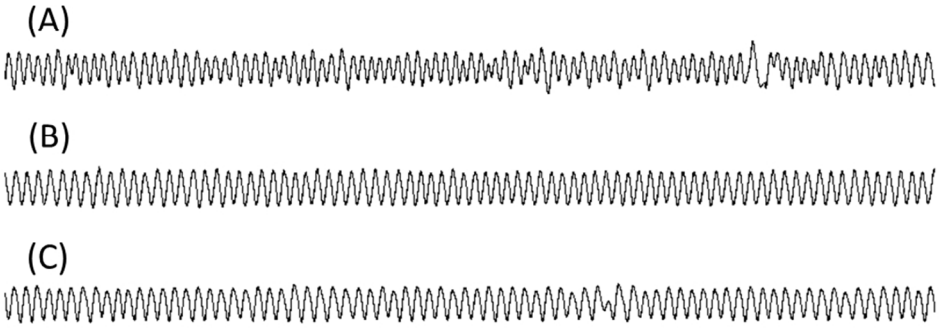
Figure legends

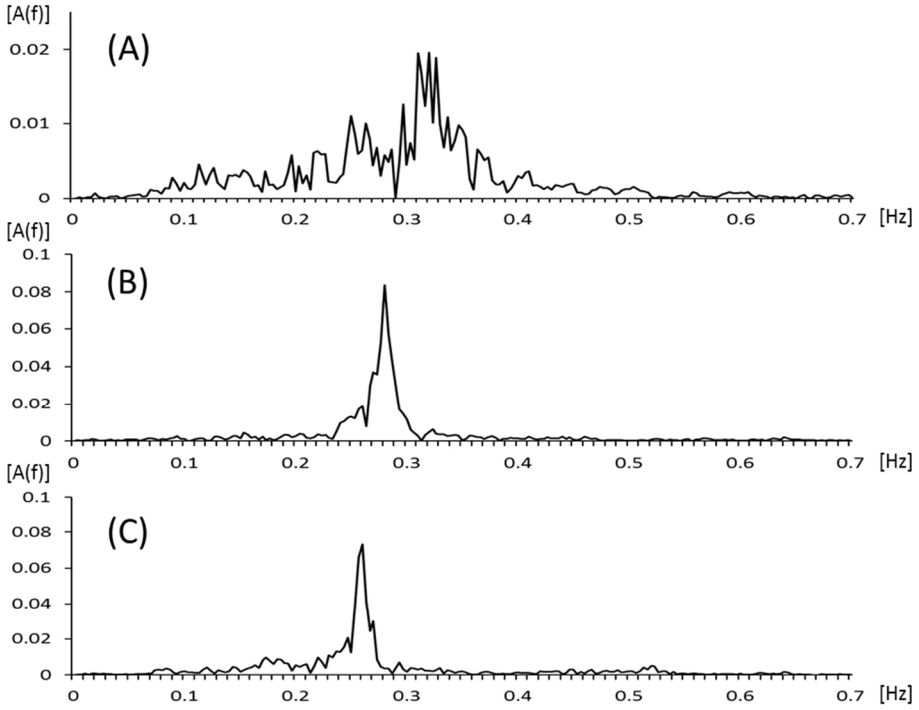
Figure 1 – Typical flow patterns of a DLB patient (A), an AD patient (B) and a non-demented patient (C) observed in epochs of five minutes. Respiratory pattern is more irregular in the DLB patient as compared with the AD patient and the non-demented patients. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies.

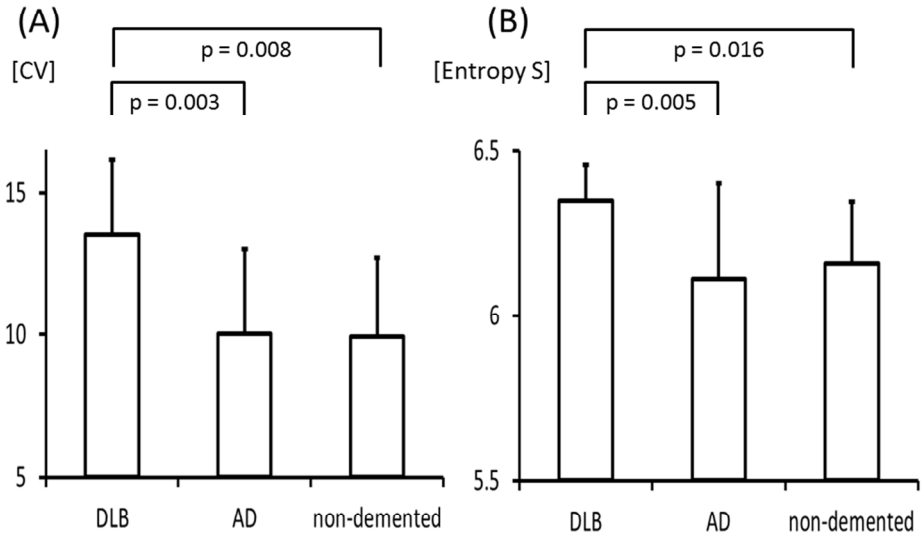
Figure 2 – The typical power spectrum of a DLB patient (A), an AD patient (B) and a non-demented patient (C) obtained by Fast Fourier Transform. The amplitude spectrum of the DLB patient is distributed over the whole displayed frequency. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; A(f) = amplitude spectrum.

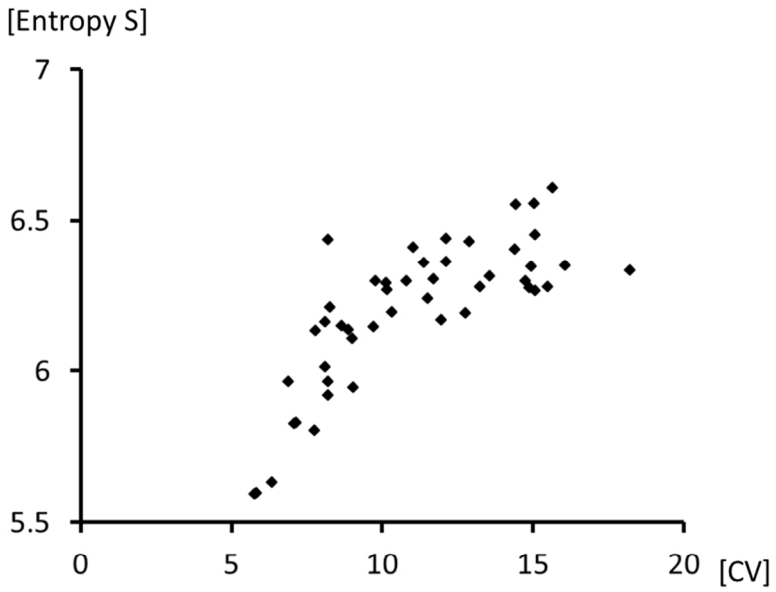
Figure 3 – (A) Coefficient of variation for breath-to-breath respiratory time in DLB patients, AD patients and non-demented patients. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD ($p = 0.003$) and DLB vs non-demented ($p = 0.008$). (B) The comparison of Shannon Entropy S in DLB patients, AD patients and non-demented subjects. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD ($p = 0.005$) and DLB vs non-demented ($p = 0.016$). Values are mean ± standard deviation. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; n.s. = not significant; CV = coefficient of variation.

Figure 4 – Scatterplot showing the relationship between the coefficient of variation for breath-to-breath respiratory time and the value of Shannon Entropy S. A significant correlation ($r = 0.78$, $p < 0.001$) was found between the CV and the Shannon Entropy S. CV = coefficient of variation.









STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Respiratory dysrhythmia in Dementia with Lewy bodies: a cross-sectional study

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Respiratory dysrhythmia in Dementia with Lewy bodies: a cross-sectional study

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Key words: ataxic breathing, dementia with Lewy bodies, Alzheimer's disease, polysomnographic recordings, Fast Fourier Transform

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Article Focus

In this study, we first indicated the presence of dysrhythmic breathing in the patients with Dementia with Lewy bodies, compared to the patients with Alzheimer's disease and the patients without dementia.

Key messages

The dysrhythmia of the breath-to-breath time on bed rest with closed eyes is significantly higher in the patients with Dementia with Lewy bodies.

The recording of the breath-to-breath time on bed rest with closed eyes can be a useful marker for the diagnosis of Dementia with Lewy bodies.

Strengths and Limitations

Dysrhythmic breathing is a completely novel topic in Dementia with Lewy bodies.

This study is a cross-sectional, small-sized pilot study.

The patholocgical diagnosis of Dementia with Lewy bodies could not be obtained.

ABSTRACT

Objectives: Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD). DLB is characterized by intracytoplasmic inclusions called Lewy bodies that are often seen in the brainstem. Because modulation of the respiratory rhythm is one of the most important functions of the brainstem, DLB patients may exhibit dysrhythmic breathing. This hypothesis has not yet been systematically studied. Therefore, we evaluated the association between DLB and dysrhythmic breathing.

Design: This was a cross-sectional study where consecutive inpatients who were admitted for the evaluation of progressive cognitive impairment were enrolled. We assessed breathing irregularity using polysomnographic recordings on bed rest with closed eyes, without reference to the clinical differentiation among DLB, AD and having no dementia.

Setting: Single center in Japan.

Participants: Fourteen DLB patients, twenty-one AD patients and twelve patients without dementia were enrolled in this study.

Primary outcome measures: The coefficient of variation (CV) of the breath-to-breath time was calculated. We also examined the amplitude spectrum A(f) obtained using the Fast Fourier Transform and Shannon Entropy S of A(f) in DLB patients compared to AD patients

and patients without dementia.

Results: The values of CV and Entropy S were significantly higher in the DLB patients than in the AD patients and patients without dementia. No significant differences were found between the AD patients and the patients without dementia.

Conclusions: DLB patients exhibit dysrhythmic breathing compared to AD patients and patients without dementia. Dysrhythmic breathing is a new clinical feature of DLB and the spectral analysis of breathing patterns can be clinically useful for the diagnostic differentiation of DLB from AD.

1. INTRODUCTION

Dementia with Lewy bodies (DLB) is a neurodegenerative disease characterized by parkinsonism, visual hallucinations and cognitive fluctuations. DLB is now thought to be the second most common form of dementia after Alzheimer's disease (AD), affecting 15-25% of elderly demented patients[1]. The clinical diagnostic criteria for DLB were first published in 1996 and modified in 2005[1,2]. The central feature of DLB is progressive cognitive decline. The core features include recurrent visual hallucinations, spontaneous features of parkinsonism and fluctuating cognition with pronounced variations in attention and alertness. These diagnostic criteria require clinical evaluation by a trained neurologist and include few objective markers. Although Single Photon Emission Computed Tomography (SPECT) and ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy are useful for making the differential diagnosis of DLB[3-5], these examinations are too expensive to be generally utilized. DLB is characterized by intracytoplasmic inclusions called Lewy bodies that consist of filamentous protein granules composed of alpha-synuclein and ubiquitin. Lewy bodies are often seen in the brainstem and in limbic and cortical neurons[2]. On the other hand, the brainstem serves as the connection among the cerebral hemispheres and the cerebellum, and is responsible for basic vital functions. Modulation of the respiratory rhythm is one of the most important functions of the brainstem. In cases of brain disorders, such as Wallenberg

syndrome and brain tumors, it is known that respiratory patterns sometimes become ataxic. Because brainstem neurodegeneration is often seen in patients with DLB, the respiratory patterns of DLB patients might be dysrhythmic. However, this hypothesis has not yet been systematically studied and no controlled data have been published to date. The present investigation was performed in patients with DLB, patients with AD and patients without dementia to assess and compare breathing patterns. In addition, we evaluated the usefulness of the measurement of breathing patterns as a novel tool to aid the differential diagnosis of dementia.

2. METHODS

2.1. Subjects

The study population comprised consecutive inpatients of the Department of Geriatric Medicine at the University of Tokyo Hospital, who were admitted for the evaluation of progressive cognitive impairment. The patients underwent neuropsychological assessments, including the Mini-Mental State Examination (MMSE), blood tests, and neuroimaging tests (Magnetic Resonance Imaging (MRI) and SPECT). The diagnosis was made at a consensus conference of physicians and neurologists. The diagnosis of DLB was based on the clinical diagnostic criteria proposed by McKeith et al in 2005[2]. And AD was diagnosed

in accordance with the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [6]. The group without dementia comprised the patients who did not fit the criteria for dementia in the medical and neurological examinations. Between November 2010 and June 2012, 70 patients were enrolled in this study.

The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

2.2. Exclusion Criteria

We evaluated the breathing patterns of DLB patients, AD patients and patients without dementia. Patients with cognitive impairments other than AD or DLB (e.g. normal pressure hydrocephalus, vascular dementia) were excluded.

Breathing irregularities are associated with certain environments such as high altitudes, medical conditions such as heart failure and chronic obstructive pulmonary disease, and the usage of opioids or levodopa[7,8]. We excluded one patient who reported breathing problems, including dyspnea. We also excluded four patients who were taking levodopa and dopamine-agonists. No patients were using opioids. We excluded three patients whose recorded respiratory signal data were insufficient due to noise.

2.3. Recordings of respiration

The patients underwent thirty minutes or more of recordings of respiration on bed rest with closed eyes in the inpatient ward by using the device for polysomnography (Somnotrac Pro, CareFusion, San Diego, CA, USA). The recordings included two electroencephalogram (EEG) leads (C3-A2 and O2-A1), electrooculogram and submental electromyogram (EMG). Oronasal thermistor channel and arterial oxygen saturation (finger oximetry) were also monitored. All recordings were scored visually by an experienced rater according to the standard criteria[9].

Five consecutive minutes of stable respiratory signals measured while the patients were awake were extracted from the recordings. Stable respiratory signals during wakefulness were identified using the respiratory signals themselves, arterial oxygen saturation, EMG and EEG. Wakefulness was confirmed using EEG. When the amplitude of the EMG signal that detected any body movements was high, that part of the signal was considered to have occurred during movement and was determined to be inappropriate for analysis. Epochs including apneas and hypopneas were also excluded.

2.4. Analysis of Respiratory Signals

Five minutes of stable respiratory signals were analyzed. The breath-to-breath time was calculated for each respiration. To assess breathing irregularities, the coefficient of variation

([standard deviation/mean] × 100) for the breath-to-breath time was calculated. The respiratory rate was also calculated.

Additionally, we examined the amplitude spectrum A(f) obtained using Fast Fourier Transform (FFT) for analyzing oscillation patterns in the respiratory signals. A(f) represents the amplitude distribution as a function of frequency. To avoid the possibility of spectral leakage, the signals were windowed by multiplying them by a Hamming window (w[n]):

$$w[n] = 0.54 - 0.46 \cos (2\pi n/N) \text{ for } n = 0,1,2,\cdots,N - 1$$

Then, the amplitude spectrum of the respiratory signals was analyzed using the FFT of the Hamming-windowed signal[10]. Furthermore, according to Shannon Entropy, we determined the spectral entropy S based on normalized A(f) in order to assess breathing irregularities:

$$\text{entropy } S = - \sum A(f) \cdot \log_2 (A(f))$$

To reduce the influence of artifact in the respiratory signals and FFT, we restricted the frequency of analyzing Shannon Entropy. Based on the results of the breath-to-breath time analysis (1.7 to 7.6 seconds, namely 0.13 to 0.59 Hz), we determined the validated frequency of 0.1 Hz to 0.6 Hz.

2.5. Statistical Analysis

The distribution of data was examined using the Shapiro-Wilk test. If data were normally

distributed, one way analysis of variance with Games-Howell post-hoc tests were applied for group comparisons. If the data deviated significantly from normality, the Kruskal-Wallis test was used, followed by evaluation with the Mann-Whitney U test for multiple comparisons, with the p values being corrected according to the Bonferroni method. In correlation analysis, the Spearman rank correlation coefficient was used. The $\chi 2$ test was used to compare categorical variables, such as gender.

The diagnostic cutoff points for the coefficient of variation value and Shannon Entropy S to discriminate between DLB and AD were estimated for each outcome by maximizing the Youden index. The discrimination ability was assessed by the area under the curve (AUC). Using this threshold, the sensitivity and specificity were calculated.

All of the statistical analyses were performed using the SPSS software program (version 19.0, SPSS inc., Chicago, IL, USA). Statistical significance was defined as P values < 0.05.

3. RESULTS

3.1. Patient Characteristics

Fourteen patients with DLB, twenty-one patients with AD and twelve patients without dementia were enrolled in this study. Among the fourteen patients in the DLB group, nine

patients had probable DLB and five patients had possible DLB. The diagnoses in the five possible DLB patients were all supported by the typical findings in SPECT: generalized low uptake, reduced occipital activity, and relatively preserved hippocampal blood flow. Table 1 shows the characteristics of the subjects. The age and sex distributions were not significantly different among the three groups. No significant difference was found between the DLB group and the AD group in the MMSE. The use of medications for hypertension, hyperlipidemia and diabetes mellitus were similar between the groups. Four patients in the DLB group, five patients in the AD group and no patients in the group without dementia had taken donepezil.

Table 1 - Characteristics of DLB patients, AD patients and patients without dementia.

Characteristics	DLB patients	AD patients	Without Dementia	p Value
Number of subjects	n = 14	n = 21	n = 12	
Age (years)	81.5 (5.6)	79.6 (7.8)	78.5 (4.3)	n.s.
Sex (men / women)	6 / 8	7 / 14	4 / 8	n.s.
MMSE	21.0 (3.8)	21.2 (3.4)	27.8 (2.1)	< 0.001*
Hypertension	4	9	3	n.s.
Hyperlipidemia	2	1	0	n.s.
Diabetes mellitus	1	1	1	n.s.

Values expressed as mean (standard deviation) or number. * = one way analysis of variance with Games-Howell post-hoc tests (DLB vs AD : n.s., DLB vs Without Dementia: $p < 0.001$, AD vs Without Dementia: $p < 0.001$); AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; MMSE = Mini-mental State Examination; n.s. = not significant.

3.2. Breathing Patterns

Figure 1 shows examples of flow signals during wakefulness for a DLB patient, an AD patient and a patient without dementia. Figure 2 shows examples of the characteristic patterns of the amplitude spectrum A(f). The AD patient and the patient without dementia exhibited a sharp peak in the spectrum. On the other hand, the amplitude spectrum of the DLB patient was distributed over the whole displayed frequency area. These tracings indicate the occurrence of more irregular breathing patterns in the DLB patient compared with that observed in the AD patient and the patient without dementia.

The respiratory rates calculated from the average breath-to-breath time in the DLB patients, the AD patients and the patients without dementia were 16.2 (3.2), 17.7 (2.7) and 18.0 (2.3) per min, respectively (mean (standard deviation)). These differences were not statistically significant. On the other hand, the coefficient of variation (CV) value for the breath-to-breath time in the DLB patients was significantly higher than that in either the AD patients or the patients without dementia (13.5 (2.6), 10.0 (3.0) and 9.9 (2.8), respectively) (Figure 3A). To discriminate the DLB patients from the AD patients using the CV value, the most favorable diagnostic threshold was found to be 10.2 (AUC = 0.79). This threshold had a sensitivity of 92.9% and a specificity of 61.9%.

The results of the comparison of Shannon Entropy S are summarized in Figure 3B. The values of Shannon Entropy S were significantly higher in the DLB patients than in the AD patients and the patients without dementia (6.35 (0.11), 6.11 (0.29) and 6.16 (0.19), respectively). To discriminate the DLB patients from the AD patients using the Shannon Entropy S value, the most favorable diagnostic threshold was found to be 6.18 (AUC = 0.77). This threshold had a sensitivity of 100% and a specificity of 57.1%.

These findings indicate the diversity of breathing frequencies, that is, respiratory dysrhythmia, in DLB patients.

3.3. Comparison of CV and Shannon Entropy S

In order to assess breathing irregularities, we used two different methods, namely, we compare CV and Shannon Entropy S. These two methods are independent approaches to the assessment of breathing patterns; however, a significant correlation (Spearman $r = 0.78$, $p < 0.001$) was observed between these two values (Figure 4).

4. DISCUSSION

In this study, we observed that patients with DLB exhibit dysrhythmic breathing compared to patients with AD and patients without dementia.

The modulation of the respiratory rhythm is closely associated with the brainstem[11]. In

particular, the pre-Bötzinger complex (pre-BötC) and the retro-trapezoid nucleus/parafacial respiratory group (RTN/pFRG) are thought to be very important for respiratory rhythm regulation[12-14]. For this reason, respiratory dysrhythmia may occur in cases of brainstem disorders, such as Wallenberg syndrome and brain tumors. In DLB patients, Lewy bodies are often seen in the brainstem; however, it remains unknown whether the localization and density of Lewy bodies are strongly associated with the symptoms of DLB. It is possible, considering the neurodegenerative aspects of DLB, that localization of Lewy bodies in the brainstem causes respiratory dysrhythmia. One report has indicated that visual hallucinations are associated with increased numbers of Lewy bodies in the temporal lobe and amygdala, each of these areas being implicated in the generation of complex visual images[15]. In addition, concerning the association between respiration and DLB, Mizukami et al. reported the occurrence of decreased ventilatory responses to hypercapnia in DLB patients[16]. Furthermore, respiratory insufficiency, sleep-disordered breathing and central respiratory failure are known to occur in patients with multiple system atrophy[17,18], which is an alpha-synucleinopathies, similar to DLB.

In this study, we also analyzed the breathing patterns of patients without dementia. The coefficient of variation for breath-to-breath time in the patients without dementia was not significantly different from that reported in previous studies of control subjects[19,20].

Although the complication with hypertension was greater in AD group than in DLB group, no significant differences were found in the measures of breathing patterns between the patients with hypertension and the patients without hypertension (data not shown).

DLB patients exhibit many clinical features other than dementia, visual hallucinations and parkinsonism. For example, Rapid Eye Movement sleep behavior disorder, severe autonomic dysfunctions such as orthostatic hypotension, repeated syncope and systematized delusions can be seen in DLB patients[21]. Furthermore, in a previous study, we reported a high frequency of periodic limb movements in DLB patients[22]. The results of the present study indicating that DLB patients exhibit dysrhythmic breathing compared to normal subjects suggest that irregular breathing patterns may be a new clinical feature of DLB.

Currently, DLB and AD are diagnosed according to their respective clinical diagnostic criteria[2,6], and differentiation of these two diseases is frequently difficult. Our findings of different breathing patterns between DLB and AD patients suggest the usefulness of the spectral analysis of breathing for discriminating patients with DLB from those with AD. Because the diagnostic threshold had a high sensitivity in our study, the spectral analysis of breathing may be useful for making an exclusive diagnosis. While the utilization of SPECT

and MIBG myocardial scintigraphy are limited to well-equipped hospitals, the spectral analysis of breathing can be performed more easily and with lower expenses. As a screening tool for the diagnosis of DLB, the spectral analysis of breathing patterns may be cost-effective and useful.

The FFT is an important tool for digital signal processing of the information commonly encoded in the sinusoids that form the signal. Additionally, the important information to be evaluated is the frequency and amplitude of the component sinusoids. To reduce spectral noise, a Hamming window is used that involves the multiplication of the signal by a smooth curve. The result is plotted graphically in terms of amplitude and frequency. In addition, we used Shannon Entropy in this study to quantify the variability of the amplitude spectrum, namely breathing irregularities. This measure has been widely used in a range of biological applications where quantitative descriptions of data regularity are required[23,24]. The Shannon Entropy indicates the degree of uncertainty and is higher when the variability of the parameter is greater.

There are several limitations to the present study. First, we included patients with possible DLB and probable DLB in the same DLB group. Additionally, we did not make a pathological diagnosis of DLB or AD. A prospective investigation on the course of breathing patterns and cognitive impairment, including the eventual pathological diagnosis, should be

examined in a future study. Second, no arterial blood gas analyses were performed. Therefore, a possible effect of hypercapnia or hypocapnia on breathing cannot be excluded. To evaluate more precisely, arterial blood gas analyses should be examined in a future study, as well. Third, we could not make the raters of respiratory measures completely blinded to the clinical symptoms of the patients, although the final diagnosis of dementia had been made independently, and the analysis of respiratory measures had been performed objectively according to the pre-determined protocol. Finally, the number of patients in each group was relatively small. We could not rule out the contribution of other comorbid factors to irregular breathing. However, our data provide the first evidence of irregular breathing in DLB patients. In a future study, an additional investigation involving a larger number of subjects should be performed.

In conclusion, we found that DLB patients exhibit dysrhythmic breathing compared to that observed in AD patients and patients without dementia. Ataxic breathing may be a new clinical feature of DLB, and the spectral analysis of breathing patterns may be clinically useful for the diagnostic differentiation of DLB from AD.

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Declaration of Interest: This was not an industry supported study. The authors have indicated no financial conflicts of interest.

Authorship responsibility: Shinichiro Hibi was involved in design, analysis, interpretation, and drafting of article. Yasuhiro Yamaguchi was responsible for conception, design, analysis, interpretation, and drafting of article. Yumi Umeda-Kameyama and Katsuya Iijima were involved in design. Miwako Takahashi and Toshimitsu Momose were involved in analysis. Masahiro Akishita and Yasuyoshi Ouchi were involved in design and interpretation. All authors had full access to the data and take responsibility for its integrity and the accuracy of the analysis.

Ethics of investigation: The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

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Figure legends

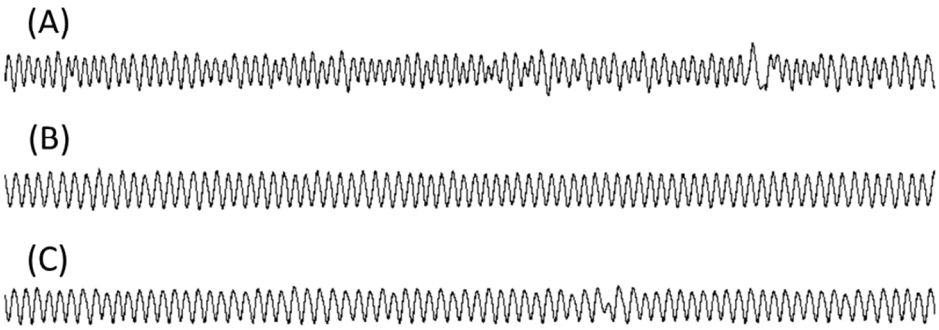
Figure 1 – Typical flow patterns of a DLB patient (A), an AD patient (B) and a patient without dementia (C) observed in epochs of five minutes. Respiratory pattern is more irregular in the DLB patient as compared with the AD patient and the patient without dementia. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies.

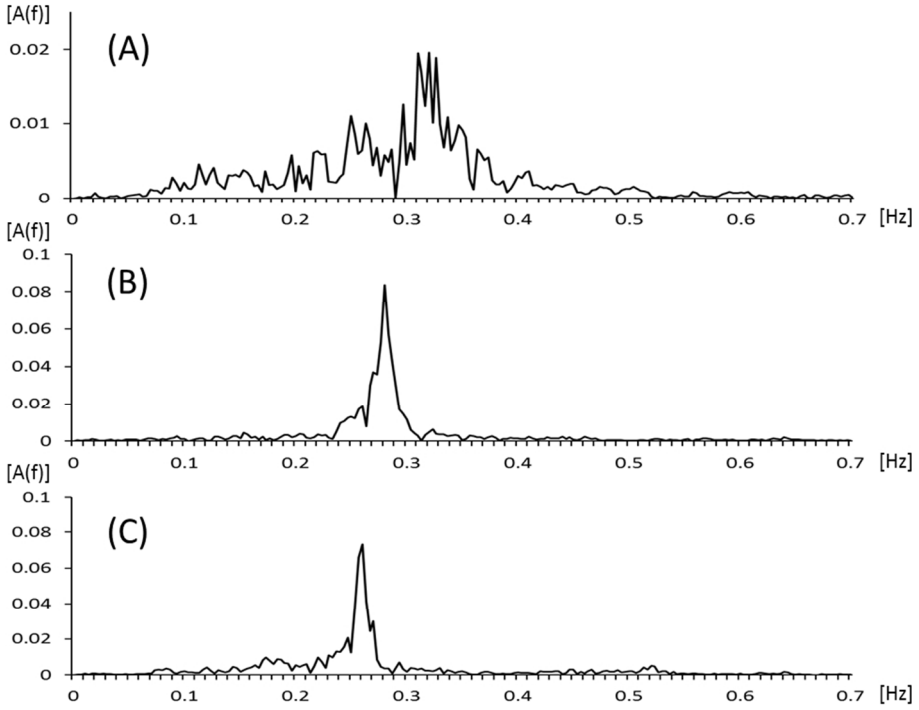
Figure 2 – The typical power spectrum of a DLB patient (A), an AD patient (B) and a patient without dementia (C) obtained by Fast Fourier Transform. The amplitude spectrum of the DLB patient is distributed over the whole displayed frequency. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; A(f) = amplitude spectrum.

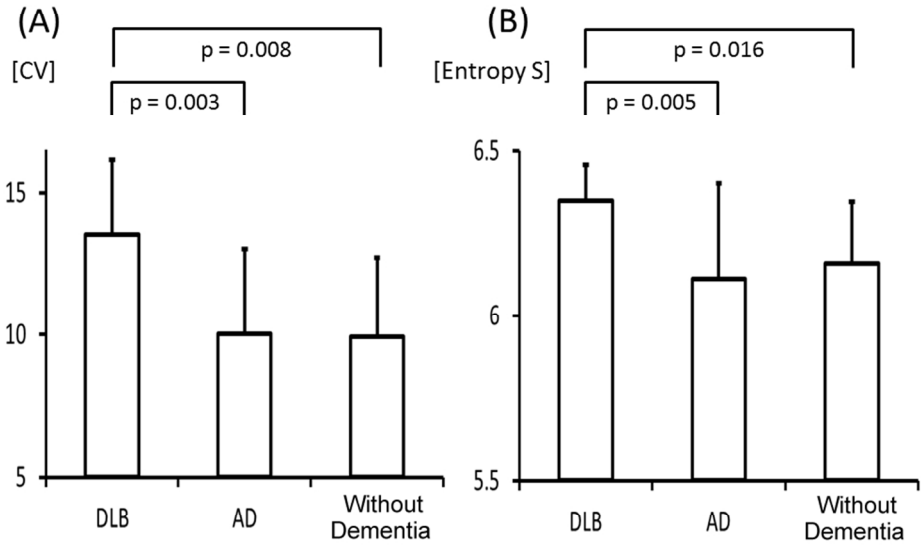
Figure 3 – (A) Coefficient of variation for breath-to-breath respiratory time in DLB patients, AD patients and patients without dementia. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD ($p = 0.003$) and DLB vs Without Dementia ($p = 0.008$). (B) The comparison of Shannon Entropy S in DLB patients, AD patients and subjects without dementia. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD ($p = 0.005$) and DLB vs Without Dementia ($p = 0.016$). Values are mean ± standard deviation. AD = Alzheimer's

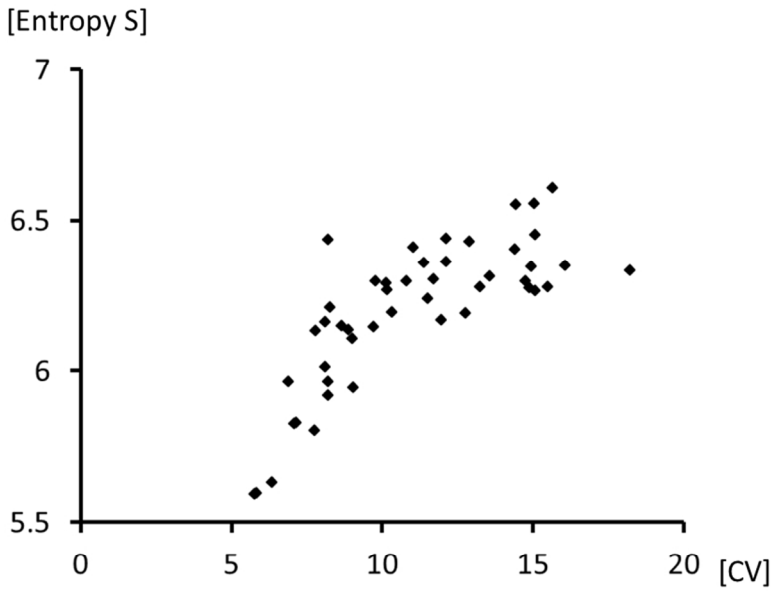
disease; DLB = Dementia with Lewy bodies; n.s. = not significant; CV = coefficient of variation.

Figure 4 – Scatterplot showing the relationship between the coefficient of variation for breath-to-breath respiratory time and the value of Shannon Entropy S. A significant correlation ($r = 0.78$, $p < 0.001$) was found between the CV and the Shannon Entropy S. CV = coefficient of variation.









STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Respiratory dysrhythmia in Dementia with Lewy bodies: a cross-sectional study

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Article Focus

In this study, we first indicated the presence of dysrhythmic breathing in the patients with Dementia with Lewy bodies, compared to the patients with Alzheimer's disease and the patients without dementia.

Key messages

The dysrhythmia of the breath-to-breath time on bed rest with closed eyes is significantly higher in the patients with Dementia with Lewy bodies.

The recording of the breath-to-breath time on bed rest with closed eyes can be a useful marker for the diagnosis of Dementia with Lewy bodies.

Strengths and Limitations

Dysrhythmic breathing is a completely novel topic in Dementia with Lewy bodies.

This study is a cross-sectional, small-sized pilot study.

The patholocgical diagnosis of Dementia with Lewy bodies could not be obtained.

ABSTRACT

Objectives: Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD). DLB is characterized by intracytoplasmic inclusions called Lewy bodies that are often seen in the brainstem. Because modulation of the respiratory rhythm is one of the most important functions of the brainstem, DLB patients may exhibit dysrhythmic breathing. This hypothesis has not yet been systematically studied. Therefore, we evaluated the association between DLB and dysrhythmic breathing.

Design: This was a cross-sectional study where consecutive inpatients who were admitted for the evaluation of progressive cognitive impairment were enrolled. We assessed breathing irregularity using polysomnographic recordings on bed rest with closed eyes, without reference to the clinical differentiation among DLB, AD and having no dementia.

Setting: Single center in Japan.

Participants: Fourteen DLB patients, twenty-one AD patients and twelve ~~non-demented~~ patients without dementia were enrolled in this study.

Primary outcome measures: The coefficient of variation (CV) of the breath-to-breath time was calculated. We also examined the amplitude spectrum A(f) obtained using the Fast Fourier Transform and Shannon Entropy S of A(f) in DLB patients compared to AD patients

and ~~non-demented~~ patients without dementia.

Results: The values of CV and Entropy S were significantly higher in the DLB patients than in the AD patients and ~~non-demented~~ patients without dementia. No significant differences were found between the AD patients and the ~~non-demented~~ patients without dementia.

Conclusions: DLB patients exhibit dysrhythmic breathing compared to AD patients and ~~non-demented~~ patients without dementia. Dysrhythmic breathing is a new clinical feature of DLB and the spectral analysis of breathing patterns can be clinically useful for the diagnostic differentiation of DLB from AD.

1. INTRODUCTION

Dementia with Lewy bodies (DLB) is a neurodegenerative disease characterized by parkinsonism, visual hallucinations and cognitive fluctuations. DLB is now thought to be the second most common form of dementia after Alzheimer's disease (AD), affecting 15-25% of elderly demented patients[1]. The clinical diagnostic criteria for DLB were first published in 1996 and modified in 2005[1,2]. The central feature of DLB is progressive cognitive decline. The core features include recurrent visual hallucinations, spontaneous features of parkinsonism and fluctuating cognition with pronounced variations in attention and alertness. These diagnostic criteria require clinical evaluation by a trained neurologist and include few objective markers. Although Single Photon Emission Computed Tomography (SPECT) and ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy are useful for making the differential diagnosis of DLB[3-5], these examinations are too expensive to be generally utilized. DLB is characterized by intracytoplasmic inclusions called Lewy bodies that consist of filamentous protein granules composed of alpha-synuclein and ubiquitin. Lewy bodies are often seen in the brainstem and in limbic and cortical neurons[2]. On the other hand, the brainstem serves as the connection among the cerebral hemispheres and the cerebellum, and is responsible for basic vital functions. Modulation of the respiratory rhythm is one of the most important functions of the brainstem. In cases of brain disorders, such as Wallenberg

syndrome and brain tumors, it is known that respiratory patterns sometimes become ataxic. Because brainstem neurodegeneration is often seen in patients with DLB, the respiratory patterns of DLB patients might be dysrhythmic. However, this hypothesis has not yet been systematically studied and no controlled data have been published to date. The present investigation was performed in patients with DLB, patients with AD and ~~non-demented~~ patients without dementia to assess and compare breathing patterns. In addition, we evaluated the usefulness of the measurement of breathing patterns as a novel tool to aid the differential diagnosis of dementia.

2. METHODS

2.1. Subjects

The study population comprised consecutive inpatients of the Department of Geriatric Medicine at the University of Tokyo Hospital, who were admitted for the evaluation of progressive cognitive impairment. The patients underwent neuropsychological assessments, including the Mini-Mental State Examination (MMSE), blood tests, and neuroimaging tests (Magnetic Resonance Imaging (MRI) and SPECT). The diagnosis was made at a consensus conference of physicians and neurologists. The diagnosis of DLB was based on the clinical diagnostic criteria proposed by McKeith et al in 2005[2]. And AD was diagnosed

in accordance with the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [6]. The ~~non-demented~~ group without dementia comprised the patients who did not fit the criteria for dementia in the medical and neurological examinations. Between November 2010 and June 2012, 70 patients were enrolled in this study.

The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

2.2. Exclusion Criteria

We evaluated the breathing patterns of DLB patients, AD patients and ~~non-demented~~ patients without dementia. Patients with cognitive impairments other than AD or DLB (e.g. normal pressure hydrocephalus, vascular dementia) were excluded.

Breathing irregularities are associated with certain environments such as high altitudes, medical conditions such as heart failure and chronic obstructive pulmonary disease, and the usage of opioids or levodopa[7,8]. We excluded one patient who reported breathing problems, including dyspnea. We also excluded four patients who were taking levodopa and dopamine-agonists. No patients were using opioids. We excluded three patients whose recorded respiratory signal data were insufficient due to noise.

2.3. Recordings of respiration

The patients underwent thirty minutes or more of recordings of respiration on bed rest with closed eyes in the inpatient ward by using the device for polysomnography (Somnotrac Pro, CareFusion, San Diego, CA, USA). The recordings included two electroencephalogram (EEG) leads (C3-A2 and O2-A1), electrooculogram and submental electromyogram (EMG). Oronasal thermistor channel and arterial oxygen saturation (finger oximetry) were also monitored. All recordings were scored visually by an experienced rater according to the standard criteria[9].

Five consecutive minutes of stable respiratory signals measured while the patients were awake were extracted from the recordings. Stable respiratory signals during wakefulness were identified using the respiratory signals themselves, arterial oxygen saturation, EMG and EEG. Wakefulness was confirmed using EEG. When the amplitude of the EMG signal that detected any body movements was high, that part of the signal was considered to have occurred during movement and was determined to be inappropriate for analysis. Epochs including apneas and hypopneas were also excluded.

2.4. Analysis of Respiratory Signals

Five minutes of stable respiratory signals were analyzed. The breath-to-breath time was calculated for each respiration. To assess breathing irregularities, the coefficient of variation

([standard deviation/mean] × 100) for the breath-to-breath time was calculated. The respiratory rate was also calculated.

Additionally, we examined the amplitude spectrum A(f) obtained using Fast Fourier Transform (FFT) for analyzing oscillation patterns in the respiratory signals. A(f) represents the amplitude distribution as a function of frequency. To avoid the possibility of spectral leakage, the signals were windowed by multiplying them by a Hamming window (w[n]):

$$w[n] = 0.54 - 0.46 \cos(2\pi n/N) \text{ for } n = 0,1,2,\cdots,N-1$$

Then, the amplitude spectrum of the respiratory signals was analyzed using the FFT of the Hamming-windowed signal[10]. Furthermore, according to Shannon Entropy, we determined the spectral entropy S based on normalized A(f) in order to assess breathing irregularities:

$$\text{entropy } S = -\sum A(f) \cdot \log_2 (A(f))$$

To reduce the influence of artifact in the respiratory signals and FFT, we restricted the frequency of analyzing Shannon Entropy. Based on the results of the breath-to-breath time analysis (1.7 to 7.6 seconds, namely 0.13 to 0.59 Hz), we determined the validated frequency of 0.1 Hz to 0.6 Hz.

2.5. Statistical Analysis

The distribution of data was examined using the Shapiro-Wilk test. If data were normally

distributed, one way analysis of variance with Games-Howell post-hoc tests were applied for group comparisons. If the data deviated significantly from normality, the Kruskal-Wallis test was used, followed by evaluation with the Mann-Whitney U test for multiple comparisons, with the p values being corrected according to the Bonferroni method. In correlation analysis, the Spearman rank correlation coefficient was used. The $\chi 2$ test was used to compare categorical variables, such as gender.

The diagnostic cutoff points for the coefficient of variation value and Shannon Entropy S to discriminate between DLB and AD were estimated for each outcome by maximizing the Youden index. The discrimination ability was assessed by the area under the curve (AUC). Using this threshold, the sensitivity and specificity were calculated.

All of the statistical analyses were performed using the SPSS software program (version 19.0, SPSS inc., Chicago, IL, USA). Statistical significance was defined as P values < 0.05.

3. RESULTS

3.1. Patient Characteristics

Fourteen patients with DLB, twenty-one patients with AD and twelve ~~non-demented~~ patients without dementia were enrolled in this study. Among the fourteen patients in the

DLB group, nine patients had probable DLB and five patients had possible DLB. The diagnoses in the five possible DLB patients were all supported by the typical findings in SPECT: generalized low uptake, reduced occipital activity, and relatively preserved hippocampal blood flow. Table 1 shows the characteristics of the subjects. The age and sex distributions were not significantly different among the three groups. No significant difference was found between the DLB group and the AD group in the MMSE. The use of medications for hypertension, hyperlipidemia and diabetes mellitus were similar between the groups. Four patients in the DLB group, five patients in the AD group and no patients in the ~~non-demented~~ group without dementia had taken donepezil.

Table 1 - Characteristics of DLB patients, AD patients and ~~non-demented~~ patients without dementia.

Characteristics	DLB patients	AD patients	Without Dementia~~Non-demented~~	p Value
Number of subjects	n = 14	n = 21	n = 12	
Age (years)	81.5 (5.6)	79.6 (7.8)	78.5 (4.3)	n.s.
Sex (men / women)	6 / 8	7 / 14	4 / 8	n.s.
MMSE	21.0 (3.8)	21.2 (3.4)	27.8 (2.1)	< 0.001*
Hypertension	4	9	3	n.s.
Hyperlipidemia	2	1	0	n.s.
Diabetes mellitus	1	1	1	n.s.

Values expressed as mean (standard deviation) or number. * = one way analysis of variance with Games-Howell post-hoc tests (DLB vs AD : n.s., DLB vs ~~Non-demented~~ Without Dementia: $p < 0.001$, AD vs Without Dementia~~Non-demented~~ : $p < 0.001$); AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; MMSE = Mini-mental State Examination; n.s. = not significant.

3.2. Breathing Patterns

Figure 1 shows examples of flow signals during wakefulness for a DLB patient, an AD patient and a ~~non-demented~~ patient without dementia. Figure 2 shows examples of the characteristic patterns of the amplitude spectrum A(f). The AD patient and the ~~non-demented~~ patient~~s~~ without dementia exhibited a sharp peak in the spectrum. On the other hand, the amplitude spectrum of the DLB patient was distributed over the whole displayed frequency area. These tracings indicate the occurrence of more irregular breathing patterns in the DLB patient compared with that observed in the AD patient and the ~~non-demented~~ patient without dementia~~s~~.

The respiratory rates calculated from the average breath-to-breath time in the DLB patients, the AD patients and the ~~non-demented~~ patients without dementia were 16.2 (3.2), 17.7 (2.7) and 18.0 (2.3) per min, respectively (mean (standard deviation)). These differences were not statistically significant. On the other hand, the coefficient of variation (CV) value for the breath-to-breath time in the DLB patients was significantly higher than that in either the AD patients or the ~~non-demented~~ patients without dementia (13.5 (2.6), 10.0

(3.0) and 9.9 (2.8), respectively) (Figure 3A). To discriminate the DLB patients from the AD patients using the CV value, the most favorable diagnostic threshold was found to be 10.2 (AUC = 0.79). This threshold had a sensitivity of 92.9% and a specificity of 61.9%.

The results of the comparison of Shannon Entropy S are summarized in Figure 3B. The values of Shannon Entropy S were significantly higher in the DLB patients than in the AD patients and the ~~non-demented~~ patients without dementia (6.35 (0.11), 6.11 (0.29) and 6.16 (0.19), respectively). To discriminate the DLB patients from the AD patients using the Shannon Entropy S value, the most favorable diagnostic threshold was found to be 6.18 (AUC = 0.77). This threshold had a sensitivity of 100% and a specificity of 57.1%.

These findings indicate the diversity of breathing frequencies, that is, respiratory dysrhythmia, in DLB patients.

3.3. Comparison of CV and Shannon Entropy S

In order to assess breathing irregularities, we used two different methods, namely, we compare CV and Shannon Entropy S. These two methods are independent approaches to the assessment of breathing patterns; however, a significant correlation (Spearman $r = 0.78$, $p < 0.001$) was observed between these two values (Figure 4).

4. DISCUSSION

In this study, we observed that patients with DLB exhibit dysrhythmic breathing compared to patients with AD and ~~non-demented~~ patients without dementia.

The modulation of the respiratory rhythm is closely associated with the brainstem[11]. In particular, the pre-Bötzinger complex (pre-BötC) and the retro-trapezoid nucleus/parafacial respiratory group (RTN/pFRG) are thought to be very important for respiratory rhythm regulation[12~~1~~-14~~3~~]. For this reason, respiratory dysrhythmia may occur in cases of brainstem disorders, such as Wallenberg syndrome and brain tumors. In DLB patients, Lewy bodies are often seen in the brainstem; however, it remains unknown whether the localization and density of Lewy bodies are strongly associated with the symptoms of DLB. It is possible, considering the neurodegenerative aspects of DLB, that localization of Lewy bodies in the brainstem causes respiratory dysrhythmia. One report has indicated that visual hallucinations are associated with increased numbers of Lewy bodies in the temporal lobe and amygdala, each of these areas being implicated in the generation of complex visual images[15~~4~~]. In addition, concerning the association between respiration and DLB, Mizukami et al. reported the occurrence of decreased ventilatory responses to hypercapnia in DLB patients[16~~5~~]. Furthermore, respiratory insufficiency, sleep-disordered breathing and central respiratory failure are known to occur in patients with multiple system atrophy[17~~6~~,18~~7~~], which is an alpha-synucleinopathies, similar to DLB.

In this study, we also analyzed the breathing patterns of ~~non-demented~~ patients without dementia. The coefficient of variation for breath-to-breath time in the ~~non-demented~~ patients without dementia was not significantly different from that reported in previous studies of control subjects[19~~8~~,20~~19~~]. Although the complication with hypertension was greater in AD group than in DLB group, no significant differences were found in the measures of breathing patterns between the patients with hypertension and the patients without hypertension (data not shown).

DLB patients exhibit many clinical features other than dementia, visual hallucinations and parkinsonism. For example, Rapid Eye Movement sleep behavior disorder, severe autonomic dysfunctions such as orthostatic hypotension, repeated syncope and systematized delusions can be seen in DLB patients[21]. Furthermore, in a previous study, we reported a high frequency of periodic limb movements in DLB patients[22~~0~~]. The results of the present study indicating that DLB patients exhibit dysrhythmic breathing compared to normal subjects suggest that irregular breathing patterns may be a new clinical feature of DLB.

Currently, DLB and AD are diagnosed according to their respective clinical diagnostic criteria[2,6], and differentiation of these two diseases is frequently difficult. Our findings of different breathing patterns between DLB and AD patients suggest the usefulness of the

spectral analysis of breathing for discriminating patients with DLB from those with AD. Because the diagnostic threshold had a high sensitivity in our study, the spectral analysis of breathing may be useful for making an exclusive diagnosis. While the utilization of SPECT and MIBG myocardial scintigraphy are limited to well-equipped hospitals, the spectral analysis of breathing can be performed more easily and with lower expenses. As a screening tool for the diagnosis of DLB, the spectral analysis of breathing patterns may be cost-effective and useful.

The FFT is an important tool for digital signal processing of the information commonly encoded in the sinusoids that form the signal. Additionally, the important information to be evaluated is the frequency and amplitude of the component sinusoids. To reduce spectral noise, a Hamming window is used that involves the multiplication of the signal by a smooth curve. The result is plotted graphically in terms of amplitude and frequency. In addition, we used Shannon Entropy in this study to quantify the variability of the amplitude spectrum, namely breathing irregularities. This measure has been widely used in a range of biological applications where quantitative descriptions of data regularity are required[23~~1~~,24~~2~~]. The Shannon Entropy indicates the degree of uncertainty and is higher when the variability of the parameter is greater.

There are several limitations to the present study. First, we included patients with

possible DLB and probable DLB in the same DLB group. Additionally, we did not make a pathological diagnosis of DLB or AD. A prospective investigation on the course of breathing patterns and cognitive impairment, including the eventual pathological diagnosis, should be examined in a future study. Second, no arterial blood gas analyses were performed. Therefore, a possible effect of hypercapnia or hypocapnia on breathing cannot be excluded. To evaluate more precisely, arterial blood gas analyses should be examined in a future study, as well. Third, we could not make the raters of respiratory measures completely blinded to the clinical symptoms of the patients, although the final diagnosis of dementia had been made independently, and the analysis of respiratory measures had been performed objectively according to the pre-determined protocol. Finally~~Third~~, the number of patients in each group was relatively small. We could not rule out the contribution of other comorbid factors to irregular breathing. However, our data provide the first evidence of irregular breathing in DLB patients. In a future study, an additional investigation involving a larger number of subjects should be performed.

In conclusion, we found that DLB patients exhibit dysrhythmic breathing compared to that observed in AD patients and ~~non-demented~~ patients without dementia. Ataxic breathing may be a new clinical feature of DLB, and the spectral analysis of breathing patterns may be clinically useful for the diagnostic differentiation of DLB from AD.

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Authorship responsibility: Shinichiro Hibi was involved in design, analysis, interpretation, and drafting of article. Yasuhiro Yamaguchi was responsible for conception, design, analysis, interpretation, and drafting of article. Yumi Umeda-Kameyama and Katsuya Iijima were involved in design. Miwako Takahashi and Toshimitsu Momose were involved in analysis. Masahiro Akishita and Yasuyoshi Ouchi were involved in design and interpretation. All authors had full access to the data and take responsibility for its integrity and the accuracy of the analysis.

Ethics of investigation: The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

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Figure legends

Figure 1 – Typical flow patterns of a DLB patient (A), an AD patient (B) and a ~~non-demented~~ patient without dementia (C) observed in epochs of five minutes. Respiratory pattern is more irregular in the DLB patient as compared with the AD patient and the ~~non-demented~~ patient~~s~~ without dementia. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies.

Figure 2 – The typical power spectrum of a DLB patient (A), an AD patient (B) and a ~~non-demented~~ patient without dementia (C) obtained by Fast Fourier Transform. The amplitude spectrum of the DLB patient is distributed over the whole displayed frequency. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; A(f) = amplitude spectrum.

Figure 3 – (A) Coefficient of variation for breath-to-breath respiratory time in DLB patients, AD patients and ~~non-demented~~ patients without dementia. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD (p = 0.003) and DLB vs ~~non-demented~~Without Dementia (p = 0.008). (B) The comparison of Shannon Entropy S in DLB patients, AD patients and ~~non-demented~~ subjects without dementia. One way analysis of variance with Games-Howell post-hoc tests; significant differences in DLB vs AD (p = 0.005) and DLB vs Without Dementia~~non-demented~~ (p = 0.016). Values are mean ±

standard deviation. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; n.s. = not significant; CV = coefficient of variation.

Figure 4 – Scatterplot showing the relationship between the coefficient of variation for breath-to-breath respiratory time and the value of Shannon Entropy S. A significant correlation ($r = 0.78$, $p < 0.001$) was found between the CV and the Shannon Entropy S. CV = coefficient of variation.