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Autopsy Validation of the Diagnostic Accuracy of ^{123}I -Metaiodobenzylguanidine Myocardial Scintigraphy for Lewy Body Disease

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Handling Editor Statement:**Abstract**

Background and Objectives: ^{123}I -meta-iodobenzyl-guanidine (^{123}I -MIBG) myocardial scintigraphy is employed as a diagnostic imaging test to differentiate Lewy body diseases (LBDs), including Parkinson's disease and dementia with Lewy bodies, from other similar diseases. However, its diagnostic accuracy lacks validation compared with that of the gold standard. We aimed to validate the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy for LBD against autopsy, the gold standard.

Methods: This retrospective, cross-sectional study included consecutive autopsy patients from the Brain Bank for Aging Research who had undergone ^{123}I -MIBG myocardial scintigraphy. We compared the ^{123}I -MIBG myocardial scintigraphy findings with autopsy findings. Furthermore, the proportion of residual tyrosine hydroxylase (TH)-immunoreactive sympathetic fibers in the anterior wall of the left ventricle was investigated to assess the condition of the cardiac sympathetic nerves assumed to cause reduced ^{123}I -MIBG uptake in LBDs.

Results: We analyzed the data of 56 patients (30 with pathologically confirmed LBDs and 26 without LBD pathology). Compared with the neuropathological diagnosis, the early heart-to-mediastinum (H/M) ratio had a sensitivity and specificity of 70.0% (95% confidence

interval [CI]: 50.6–85.3%) and 96.2% (95% CI: 80.4–99.9%), respectively. The delayed H/M ratio had a sensitivity and specificity of 80.0% (95% CI: 61.4–92.3%) and 92.3% (95% CI: 74.9–99.1%), respectively. The washout rate had a sensitivity and specificity of 80.0% (95% CI: 61.4–92.3%) and 84.6% (95% CI: 65.1–95.6%), respectively. The proportion of residual TH-immunoreactive cardiac sympathetic fibers strongly correlated with the amount of cardiac ¹²³I-MIBG uptake when assessed with early and delayed H/M ratio values (correlation coefficient: 0.75 and 0.81, respectively; $p < 0.001$).

Discussion: This clinicopathological validation study revealed that ¹²³I-MIBG myocardial scintigraphy could robustly differentiate LBDs from similar diseases. Abnormal ¹²³I-MIBG myocardial scintigraphy findings strongly support the presence of LBD and cardiac sympathetic denervation. However, LBD pathology should not necessarily be excluded by normal myocardial scintigraphy results, especially when other biomarkers suggest the presence of comorbid Alzheimer's disease pathology.

Classification of Evidence: This study provides Class II evidence that ¹²³I-MIBG myocardial scintigraphy accurately identifies patients with LBD.

Introduction

Lewy body disease (LBD) is characterized by the presence of Lewy bodies, composed of α -synuclein, and it encompasses a diagnostic spectrum, including Parkinson's disease (PD) and dementia with Lewy bodies (DLB).^{1, 2} Accurately diagnosing LBDs and differentiating them from other similar diseases are critical for both patient care and research. To date, various clinical tools have been developed to aid LBD diagnosis. The detection of reduced cardiac uptake of ^{123}I -meta-iodobenzyl-guanidine (^{123}I -MIBG), a physiological analog of norepinephrine,³ has been used as a diagnostic imaging tool (specifically in ^{123}I -MIBG myocardial scintigraphy) to differentiate LBDs from other similar diseases.^{1, 4} This test is based on the fact that LBD pathology accompanied by denervation is observed not only in the central nervous system (CNS) but also in the peripheral nervous system (PNS), including the cardiac sympathetic nerves.⁵⁻¹¹

Several studies have evaluated the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy for LBDs.¹²⁻¹⁶ Those studies, however, used the clinical diagnosis as a reference standard for the evaluation of the diagnostic accuracy. Despite the current development of diagnostic biomarkers, neuropathological confirmation remains the gold standard for LBD diagnosis. Several pathological investigations have highlighted the discrepancy between clinical diagnoses and postmortem pathological diagnoses in LBDs and similar neurodegenerative diseases.¹⁷⁻²⁴ Therefore, validation of the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy against the gold standard is required.^{12, 14}

Here, using a large autopsy series, we determined the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy in differentiating LBDs from similar conditions. Furthermore, we performed a neuropathological investigation to examine the factors contributing to false-positive or false-negative ^{123}I -MIBG myocardial scintigraphy results.

Methods

Study Design

We performed a retrospective, cross-sectional study to evaluate the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy for the differential diagnosis of LBD and similar diseases, against the gold standard of neuropathological examination.

Standard Protocol Approvals, Registrations, and Patient Consent

Our study was approved by the local institutional ethics committee (approval number: R20-022). Written informed consent was obtained from the patients' families prior to the autopsy. This study was performed in accordance with the principles of the Declaration of Helsinki, and the manuscript was structured according to the STARD (STAndards for Reporting of Diagnostic accuracy) statement 2015.^{e1}

Participants and Settings

The study included consecutive patients autopsied at the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology between January 2006 and February 2021. The hospital is located in a suburban area of Tokyo, Japan, and provides community-based general and emergency services for the elderly population, including patients with dementia or neurodegenerative diseases.

We included patients who underwent ^{123}I -MIBG myocardial scintigraphy and applied the following exclusion criteria: (1) undergoing ^{123}I -MIBG myocardial scintigraphy for prognosis prediction of heart failure,^{25, 26} (2) using medications that strongly affect ^{123}I -MIBG myocardial scintigraphy results (tricyclic antidepressants, serotonin noradrenaline reuptake inhibitor, labetalol, reserpine, and phenylephrine),²⁷ and (3) no consent for craniotomy or registration to the Brain Bank for Aging Research (BBAR).

¹²³I-MIBG Myocardial Scintigraphy

Following the injection of 111 MBq ¹²³I-MIBG (FUJIFILM Toyama Chemical, Co., Ltd., Tokyo, Japan), early and delayed images were obtained with delays of 15–30 minutes and 3–4 hours, respectively. The scintigraphy findings were interpreted immediately and recorded; thus, these data were interpreted independently of the results of the autopsies. The heart-to-mediastinum (H/M) ratio was calculated using a standard method,²⁸ dividing the average count per pixel in the circular region of interest (ROI) on the heart by the average count per pixel in the rectangular ROI on the upper mediastinum. The participants underwent ¹²³I-MIBG myocardial scintigraphy at different periods or facilities; thus, the collimator differences were standardized using a calibration phantom or conversion coefficients established by a previous study.²⁹ Accordingly, all H/M ratios were converted to a value comparable to a medium-energy type collimator. The cutoff value used for the H/M ratios was 2.20, which is widely used.^{26, 30}

The washout rate was calculated from early and late heart counts (H_E and H_L , respectively) and mediastinal counts (M_E and M_L , respectively), using the following formula with background (mediastinal counts) and decay corrections:

$$([H_E - M_E] - [H_L - M_L]/DCF)/(H_E - M_E) \times 100(\%)$$

where DCF is a decay correction factor, calculated as $0.5^{(\text{time [h] between early and late images} / ^{123}\text{I half-life [h]})}$. The washout rate values were not standardized. The cutoff value used for the washout rate was 34%, which is widely used.^{26, 30}

Neuropathological Analysis and Diagnosis

The neuropathological analysis of both CNS and PNS was performed as previously reported.^{10, 31} Briefly, during autopsy, the brain was divided into halves; from one half, some representative parts were sampled for diagnosis and fixed with 4% paraformaldehyde for 48 hours, and the remaining parts were frozen. In addition, tissue samples were obtained from the spinal cord and PNS, including the paravertebral sympathetic ganglia (stellate or upper thoracic ganglia) and

anterior wall of the heart left ventricle, and then fixed. The other half of the brain was fixed in 20% buffered formalin for 1–2 weeks. Representative anatomical areas were sampled and embedded in paraffin, and 6- μ m thick sections were stained with hematoxylin–eosin and Klüver–Barrera, and by Gallyas–Braak silver impregnation. Subsequently, immunoreaction product deposits on immunohistochemically stained sections were visualized with a Ventana BenchMark GX autostainer (Ventana Medical Systems, Tucson, AZ, USA), an I-View Universal DAB Detection Kit (Roche, Basel, Switzerland), and primary antibodies against phosphorylated α -synuclein (pSyn#64; dilution 1:20,000 with formic acid for antigen retrieval; a gift from T. Iwatsubo, Japan; now available for purchase from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), non-phosphorylated α -synuclein (LB509; dilution 1:100 with protease K as pretreatment; a gift from T. Iwatsubo, Japan; now available for purchase from BioLegend, San Diego, CA, USA and others), phosphorylated tau (AT8; dilution 1:1,000; Innogenetics, Ghent, Belgium), human amyloid β (12B2; dilution 1:50 with formic acid for antigen retrieval; IBL, Gunma, Japan), phosphorylated TDP-43 (pSer409/410; dilution 1:10,000 with microwave in Dako target retrieval solution [pH 6.0] for antigen retrieval; a gift from M. Hasegawa, Japan; now available for purchase from Cosmo Bio, Tokyo, Japan), and tyrosine hydroxylase (TH16; dilution 1:4,000 with microwave in Dako target retrieval solution [pH 6.0] for antigen retrieval; Sigma-Aldrich, Saint Louis, MO, USA).

The cases were neuropathologically assessed in this study by 2 neuropathologists (TM and YS) blinded to the clinical data and 123 I-MIBG myocardial scintigraphy results. Neuropathological diagnoses were also assigned by employing the internationally accepted neuropathological criteria for the diagnosis of LBD,^{1, 31, 32} Alzheimer's disease (AD),³³ progressive supranuclear palsy (PSP),³⁴ multiple system atrophy (MSA),³⁵ corticobasal degeneration (CBD),³⁶ frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP),³⁷ and argyrophilic grain disease (AGD).³⁸ Because the main aim of this study was to evaluate the diagnostic accuracy of 123 I-MIBG myocardial scintigraphy for detecting LBDs, all patients with Lewy body

α -synuclein pathology, including incidental focal Lewy body α -synuclein pathology, were regarded as having LBDs.

Quantitative Analysis of the Cardiac Sympathetic Nerve

To assess the condition of the cardiac sympathetic nerves assumed to cause reduced uptake of MIBG in LBDs,^{7, 11} we investigated the sympathetic nerve in the anterior wall of the left ventricle, where cardiac MIBG uptake is mainly observed. The residual cardiac sympathetic nerve fiber area was determined with reference to previously described methods.^{9, 11, 39} Briefly, short-axis sliced nerve fascicles in the epicardium, with diameters $\geq 50 \mu\text{m}$ and maximum diameter/minimum diameter < 2 , were photographed for at least 5 fascicles per case using a digital camera (DS-Ri2, Nikon, Japan) connected to a microscope (Eclipse Ni, Nikon, Japan) with a 40 $\times$ objective lens. These hematoxylin and DAB staining images were processed using Image J/Fiji software (National Institutes of Health, Bethesda, MD, USA),^{40, e2} and areas of hematoxylin and DAB staining were divided using the “Colour Deconvolution” function.⁴¹ After binarizing the images using the threshold function, the proportion of residual tyrosine hydroxylase (TH)-immunoreactive sympathetic fibers was calculated as the TH-immunoreactive area/total endoneurium area (fascicle area).

Statistical Analysis

The analyses were performed using Stata/IC 16 (Stata Corp LP, College Station, TX, USA) and JMP Pro 15 (SAS Institute Inc., Cary, NC, USA). The normality of data was assessed using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Subsequently, comparisons were performed using the *t*-test for normally distributed continuous variables, the Mann–Whitney U test for non-normally distributed continuous variables, and the chi-square test for categorical variables. The sensitivity, specificity, positive predictive values, and negative predictive values were estimated with the 95% Clopper–Pearson exact confidence intervals (CIs) assuming binomial distribution. To evaluate optimal cutoff values, we performed receiver operating characteristic

(ROC) curve analysis and measured the area under the ROC curve (AUC) with 95% CI. The optimal cutoff values of the H/M ratios and washout rate for differentiating LBDs from non-LBDs were determined using the values that maximized sensitivity with the lower limit of the 95% CI for a specificity being set at 0.80 or higher. The correlations between the H/M ratios and the proportion of residual TH-immunoreactive sympathetic fibers were evaluated using Spearman's rank correlation coefficient. All statistical tests were two-sided, and the significance level was set at 0.05. The results are expressed as mean $\pm$ standard deviation.

Sample Size Estimation

The H/M ratios of ^{123}I -MIBG myocardial scintigraphy have an estimated specificity of 0.90 or more, based on previous studies.¹²⁻¹⁶ Furthermore, diagnostic biomarkers for LBDs require a specificity of 0.80 or higher.⁴ A sample size of 26 non-LBD patients produces a two-sided 95% CI with a width equal to 0.15 when the sample specificity is assumed to be 0.90 or more.

Data Availability Statement

The datasets and full protocol of the present study are available from the corresponding author on reasonable request.

Classification of Evidence

The primary research question was to determine the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy (in particular, the specificity) based on neuropathological examination. This study provides Class II evidence for distinguishing patients with and without LBD pathology using ^{123}I -MIBG myocardial scintigraphy.

Results

Baseline Characteristics

Autopsies were performed on 1,187 consecutive patients. We excluded 340 patients owing to the lack of consent for craniotomy and registration to BBAR, 1 with ^{123}I -MIBG myocardial scintigraphy for prognosis prediction in heart failure, 1 receiving an antidepressant at the time of ^{123}I -MIBG myocardial scintigraphy, and 789 who did not undergo ^{123}I -MIBG myocardial scintigraphy (the index test). We examined 56 patients who underwent the index test to diagnose LBDs (Figure 1). The mean age of the patients at death was 82.2 ± 9.3 years (range: 41–99 years); 51.8% ($n = 29$) were male, and 48.2% ($n = 27$) were female. The patients' characteristics are summarized in Table 1. The mean disease duration, interval from symptom onset to index test, and interval from index test to autopsy (reference standard) were 5.0 ± 4.2 years (range: 0.1–16.2 years), 3.9 ± 3.0 years (range: 0.1–12.8 years), and 8.9 ± 4.8 years (range: 0.5–19.0 years), respectively. 28 patients were clinically suspected of having LBD, and the remaining 28 were suspected of having non-LBD. No adverse events were reported since performing the index test.

Neuropathological Diagnosis

The neuropathologists were in complete agreement regarding the neuropathological diagnoses of the patients included in this study. Among the 56 patients examined in this study, 30 were classified as having LBD and 26 as non-LBD. The 30 patients with LBD were diagnosed as having PD ($n = 19$), DLB ($n = 9$; including DLB + AD $n = 5$), and incidental LBD ($n = 2$). The BBAR LB stages were 0.5 ($n = 2$), 2 ($n = 1$), 3 ($n = 6$), 4 ($n = 13$), and 5 ($n = 8$). To assess the validity of ^{123}I -MIBG myocardial scintigraphy for detecting LBDs, all patients with either pure LBD or mixed LBD were regarded as having proven LBD, and all other patients were classified as having non-LBD ($n = 26$: 10 with PSP, 3 with AD, 3 with MSA, 2 with FTLD-TDP, 2 with AGD, 1 with CBD, 1 with spinocerebellar degeneration, 1 with cerebrovascular disease, 1 with hydrocephalus, 1 with brain tumor, and 1 with minimal pathological change).

Considering the ^{123}I -MIBG myocardial scintigraphy results together with the pathological diagnoses, 24 patients with LBD had abnormal results for one or both of the H/M ratios; 24 patients without LBD had normal results for both the H/M ratios; 6 patients with LBD, including 1 with PD, 3 with DLB + AD, and 2 with incidental LBD, had normal results for both the H/M ratios; and 2 patients without LBD, including 1 with FTLT-DTP and 1 with AGD, had abnormal results for one or both of the H/M ratios.

Diagnostic Accuracy of ^{123}I -MIBG Myocardial Scintigraphy

The H/M ratio was lower in the LBD group (early: 1.86 ± 0.52 ; delayed: 1.65 ± 0.59) than in the non-LBD group (early: 3.09 ± 0.58 , $p < 0.001$; delayed: 3.04 ± 0.63 , $p < 0.001$; Figure 2A, B).

The washout rate was higher in the LBD group ($47.2 \pm 19.4\%$) than in the non-LBD group ($21.8 \pm 13.0\%$, $p < 0.001$; Figure 2C).

The cross-tabulation of the test results and data on the diagnostic accuracy of ^{123}I -MIBG myocardial scintigraphy for discriminating LBDs from non-LBDs are summarized in Table 2. Briefly, when applying the cutoff value of 2.20 to the early H/M ratio (refer to the Methods section for details), the sensitivity and specificity were 70.0% (95% CI: 50.6–85.3%) and 96.2% (95% CI: 80.4–99.9%), respectively. When applying the cutoff value of 2.20 to the delayed H/M ratio, the sensitivity and specificity were 80.0% (95% CI: 61.4–92.3%) and 92.3% (95% CI: 74.9–99.1%), respectively. When applying the cutoff value of 34% to the washout rate (refer to the Methods section for details), the sensitivity and specificity were 80.0% (95% CI: 61.4–92.3%) and 84.6% (95% CI: 65.1–95.6%), respectively.

Sensitivity Analysis

The ROC curve was used to calculate the optimal cutoff value of the ^{123}I -MIBG myocardial scintigraphy. The AUCs of the early and delayed H/M ratios and washout rate were 0.94 (95%

CI: 0.85–0.99), 0.93 (95% CI: 0.83–0.98), and 0.87 (95% CI: 0.76–0.95), respectively (Figure 3A–D).

The optimal cutoff values to maximize the sensitivity, with the lower limit of the CI for a specificity of 0.80 or higher, were calculated. When applying the cutoff value of 2.22 to the early H/M ratio, the sensitivity and specificity were 73.3% (95% CI: 54.1–87.7%) and 96.2% (95% CI: 80.4–99.9%), respectively (Figure 3B). When applying the cutoff value of 1.81 to the delayed H/M ratio, the sensitivity and specificity were 80.0% (95% CI: 61.4–92.3%) and 100% (95% CI: 86.8–100%), respectively (Figure 3C). When applying the cutoff value of 41% to the washout rate, the sensitivity and specificity were 66.7% (95% CI: 47.2–82.7%) and 96.2% (95% CI: 80.4–99.9%), respectively (Figure 3D).

Relationship Between Cardiac ^{123}I -MIBG Uptake and Cardiac Sympathetic Denervation

The cardiac TH-immunoreactive sympathetic fiber area was measured in 55 of the 56 (98.2%) patients; in 1 patient, the left ventricular anterior wall was not sampled. The TH-immunoreactive sympathetic fiber area was measured in at least 5 fascicles per patient (mean: 8.9 fascicles), and the mean proportion of residual TH-immunoreactive sympathetic fibers was calculated.

A marked loss of TH-immunoreactive sympathetic fibers was noted in patients with LBD compared with those without LBD ($p < 0.001$); the LBD and non-LBD groups showed a mean proportion of residual TH-immunoreactive sympathetic fibers of 0.15 ± 0.24 (range 0.0021–0.72) and 0.55 ± 0.14 (range 0.24–0.83), respectively. The proportion of residual TH-immunoreactive sympathetic fibers correlated with the amount of cardiac ^{123}I -MIBG uptake in the early H/M ratio (correlation coefficient [r] = 0.75, $p < 0.001$; Figure 4A) and in the delayed H/M ratio ($r = 0.81$, $p < 0.001$; Figure 4B). Typical microscopic images of true-positive, false-positive, and false-negative cases are shown in Figure 5.

Subgroup Analysis

The patients were divided into 3 subgroups (motor symptoms, dementia, or autonomic failure) according to their initial complaints to assess the validity of ^{123}I -MIBG myocardial scintigraphy for detecting LBDs in the context of movement disorders or dementia. In addition, the patients were divided into 2 subgroups to verify whether the interval between onset and ^{123}I -MIBG myocardial scintigraphy (< 3 years or ≥ 3 years) affected the diagnostic accuracy. The distribution of H/M ratios in these subgroups is shown in Figure 2D. In the motor-symptom-onset subgroup ($n = 37$), 18 patients had LBD; among these, 14 and 17 patients had abnormal early and delayed H/M ratios, respectively. Among the remaining 19 non-LBD patients, none had abnormal early or delayed H/M ratios. In the dementia-onset subgroup ($n = 17$), 10 patients had LBD, and 5 of these patients had abnormal early and delayed H/M ratios. Among the remaining 7 non-LBD patients, 1 and 2 had abnormal early and delayed H/M ratios, respectively. In the motor-symptom-onset subgroup, 3 of the 4 patients with false-negative results in the early H/M ratio, and 1 patient with false-negative results in the delayed H/M ratio were in the subgroup tested within 3 years after onset. In the dementia-onset group, all 5 patients with false-negative results for both the early and delayed H/M ratios were in the subgroup tested more than 3 years after onset; furthermore, 4 of the 5 patients had both LBD and AD pathological findings.

To assess whether specific diseases among non-LBD patients would show abnormal ^{123}I -MIBG myocardial scintigraphy results, we examined the H/M ratio values for patients with either PSP, MSA, or AD, which constituted the majority of non-LBDs in this study as well as were the primary differential diagnoses of LBD. In patients with PSP ($n = 10$), the mean early and delayed H/M ratios were 2.99 ± 0.54 (range 2.43–3.96) and 2.98 ± 0.52 (range 2.32–4.03), respectively. In patients with MSA ($n = 3$), the early and delayed H/M ratios were found to be 3.75 and 4.08; 3.11 and 3.36; and 3.69 and 4.45, respectively. Furthermore, in patients with AD without LBD (n

= 3), the early and delayed H/M ratios were found to be 3.14 and 2.34; 2.99 and 2.71; and 2.42 and 2.54, respectively.

Discussion

Our clinicopathological validation study revealed that ^{123}I -MIBG myocardial scintigraphy exhibited a robust diagnostic accuracy in differentiating LBDs from other similar diseases, demonstrating 70.0% sensitivity and 96.2% specificity for the early H/M ratio, 80.0% sensitivity and 92.3% specificity for the delayed H/M ratio, and 80.0% sensitivity and 84.6% specificity for the washout rate. These findings corroborate previously reported studies that used the clinical diagnosis by expert neurologists as the reference standard.¹²⁻¹⁶ Our results demonstrated that owing to its high specificity, ^{123}I -MIBG myocardial scintigraphy is an effective tool to enhance the diagnostic accuracy, considering that a diagnostic test with high specificity ($\geq 80\%$) is required.⁴

Furthermore, on limiting the analysis to the subgroup of patients who first presented with motor symptoms, we found that H/M ratios had a higher specificity with no false-positive results. In contrast, dopamine-transporter imaging, another widely used diagnostic aid imaging tool for LBDs, is known to be ineffective in differentiating LBD from other similar diseases presenting with parkinsonism.^{42, 43} This high specificity of ^{123}I -MIBG myocardial scintigraphy in differentiating LBDs from other similar diseases presenting with parkinsonism will strongly enhance the importance of ^{123}I -MIBG myocardial scintigraphy. Therefore, there will be situations where ^{123}I -MIBG myocardial scintigraphy becomes the decisive factor in diagnosis.

Among the indicators considered, early and delayed H/M ratios were more useful, showing large AUCs. Our study could not provide evidence of whether the washout rate was superior to the H/M ratios in terms of sensitivity, specificity, or AUC. Unlike the H/M ratios, the washout ratio has no established standardization method, thus affecting its diagnostic accuracy. Possibly,

adding the washout rate in the assessment for cases where there is a discrepancy between early and delayed H/M ratios could lead to a correct diagnosis. Indeed, when we explored this hypothesis, the washout rate results suggested the correct pathology in 3 of the 4 H/M ratio-discrepant cases in this study. Future clinical studies focusing on this hypothesis may clarify the significance of adding the washout rate to the H/M ratios.

This study corroborated the LBD diagnostic capability of ^{123}I -MIBG myocardial scintigraphy by comparing its results with the results of a systematic pathological evaluation, including quantitative analysis of the cardiac sympathetic nerve fibers. A previous study revealed that cardiac TH-immunoreactive sympathetic fibers were reduced in LBDs with a decreased H/M ratio, although the non-LBD group in that study included only a small number of patients.¹¹ With a quantitative analysis of a larger number of patients, including patients without LBD, our investigation provided further pathological evidence of the cardiac sympathetic nerve fibers in the LBD group with decreased H/M ratios in ^{123}I -MIBG myocardial scintigraphy as well as in the group with preserved H/M ratios including with or without LBD pathology. As a result, we showed a strong correlation between the H/M ratio and residual cardiac TH-immunoreactive sympathetic fibers.

A small number of patients in our study had false-positive or false-negative ^{123}I -MIBG myocardial scintigraphy results, even for the H/M ratios. In total, 6 patients had false-negative results (LBDs without decreased H/M ratio) for both the H/M ratios. In 1 patient, the interval from the onset of disease to ^{123}I -MIBG myocardial scintigraphy was as short as 1 year, while in the remaining 5 patients, ^{123}I -MIBG myocardial scintigraphy was performed more than 3 years after disease onset; of note, 4 of the 5 patients had both LBD and AD pathological findings. LBD pathology and concomitant AD pathologies are infrequently associated with the accumulation of α -synuclein and denervation in the heart.^{6, 39, 44} Our study further confirmed these findings and newly revealed that a decrease in the H/M ratios on ^{123}I -MIBG myocardial scintigraphy also

tends to occur less frequently in such conditions. Thus, the presence of the LBD pathology should not necessarily be excluded based on the normal results of ^{123}I -MIBG myocardial scintigraphy alone, especially when other biomarkers suggest the presence of comorbid AD pathology. This discrepancy should be recognized as a pitfall of ^{123}I -MIBG myocardial scintigraphy. Furthermore, two entries/pathways or multicentric occurrence have been hypothesized for LBD pathology propagation;^{45, 46} this hypothesis and infrequent peripheral LBD pathology in the presence of comorbid AD pathology suggest that the presence of AD pathology may influence the spread or distribution of LBD pathology. Moreover, all the false-negative cases in our study had similar amounts of residual cardiac TH-immunoreactive sympathetic fibers as those in the non-LBD group, even at the time of the autopsy. This finding suggests that repeating the ^{123}I -MIBG myocardial scintigraphy after a time interval is not always helpful. As per the subgroup analysis results, motor-symptom-onset LBDs were associated with false-negatives mainly in the group within 3 years of onset, while dementia-onset LBDs were associated with false-negatives even after more than 3 years from onset. Considering these observations, when false-negative results are suggested, especially in dementia-onset cases, the use of other modalities should be considered.

In contrast, 2 patients exhibited false-positive results (non-LBDs with decreased H/M ratio) for one or both of the H/M ratios; both patients had no history of cardiovascular disease or diabetes and did not take any relevant medications. One patient had FTLT-TDP, and the other had AGD. Rarely, the H/M ratio is mildly decreased in association with neurodegenerative diseases such as PSP and MSA,^{47, 48} although all the patients with PSP or MSA in the present study had normal ^{123}I -MIBG myocardial scintigraphy results. To our knowledge, there is a lack of evidence on the decreased H/M ratio in FTLT-TDP or AGD. In fact, both patients in our study had a mean proportion of residual TH-immunoreactive sympathetic fibers comparable with those of true-negative cases as well as showed no accumulation of TDP-43 protein, argyrophilic grain, or α -synuclein in the spinal cord, sympathetic ganglia, or heart. Of note, previous studies have

shown that the H/M ratios decrease with age, especially in the delayed phase,^{49, 50} and could decrease slightly below the cutoff value of 2.2 even in healthy people.⁴⁹ In fact, in a database of the Japanese Society of Nuclear Medicine working group, a healthy 64-year-old participant exhibited slightly decreased H/M ratios below the cutoff value (2.1 for the early and 2.0 for the delayed H/M ratio).⁴⁹ Certainly, both patients who had a false-positive H/M ratio in our study were elderly (aged 78 and 84 years at the time of ¹²³I-MIBG scintigraphy) and their hearts were visible, resulting in H/M ratios only slightly below the cutoff value. Our data also indicated that variations in the H/M ratio values and residual cardiac sympathetic nerve fiber among non-LBD individuals exist; thus, the cutoff threshold for LBDs should be carefully considered. The present ROC curve analysis suggests that lowering the cutoff threshold for delayed H/M ratio to 1.8 increases the specificity without reducing the sensitivity, thereby allowing enhancement of the diagnostic utility of ¹²³I-MIBG myocardial scintigraphy. Given that ¹²³I-MIBG myocardial scintigraphy cannot avoid false-negative results, lowering the cutoff threshold for the H/M ratios, especially in the delayed phase, and emphasizing specificity may be a reasonable option for LBD diagnosis.

This study has some limitations. First, although this was a large consecutive autopsy cohort study, consent for craniotomy and registration to BBAR was not obtained from 340 of 1,187 (28.6%) potentially eligible patients. However, only 5 patients underwent ¹²³I-MIBG myocardial scintigraphy among the patients without consent for craniotomy or registration to BBAR. In addition, no significant difference in patients' background was observed between patients with and without consent for craniotomy or registration to BBAR. Second, because of the study's retrospective nature, the clinical information, including clinical characteristics and neurological examination, was obtained from medical records. In particular, data on the possible presence of autonomic dysfunction were not systematically recorded. Third, this study may have a potential selection bias, although ¹²³I-MIBG myocardial scintigraphy was performed regardless of whether LBD was suspected. This is because the study mainly included patients who were seen for the

screening of dementia or movement disorders, and therefore, the study might potentially have missed some patients with very early or silent LBD who did not visit the hospital. This factor may have resulted in the slight inflation of the reported sensitivity. Finally, the intervals from ^{123}I -MIBG myocardial scintigraphy to death differed among patients, and this variability might have affected the extent of cardiac sympathetic denervation.

In conclusion, our autopsy study revealed a strong correlation between abnormal cardiac sympathetic activity, evaluated with ^{123}I -MIBG myocardial scintigraphy, and LBD diagnosis, confirming the utility of this imaging test for the diagnosis of LBDs. Altered ^{123}I -MIBG myocardial scintigraphy strongly supports the presence of LBDs. However, the presence of LBD pathology should not necessarily be excluded with normal myocardial scintigraphy results, especially when other biomarkers suggest the presence of comorbid AD pathology, and this discrepancy should be recognized as a pitfall of ^{123}I -MIBG myocardial scintigraphy. Future studies on different patient populations are needed to confirm these findings.

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eReferences e1–e2 are available at [LINK].

Table 1: Baseline demographic and clinical characteristics of participants

	All (n = 56)	LBD (n = 30)	non-LBD (n = 26)
Sex, Male: Female	29:27	17:13	12:14
Age at death (Y), mean (SD) [range]	82.2 (9.3) [41–99]	84.9 (7.5) [68–99]	79.0 (10.2) [41–91]
Interval between onset and ¹²³ I-MIBG scintigraphy (Y), mean (SD) [range]	5.0 (4.2) [0.1–16.2]	5.0 (4.7) [0.2–16.2]	5.0 (3.6) [0.1–12.3]
Interval between ¹²³ I-MIBG scintigraphy and death (Y), mean (SD) [range]	3.9 (3.0) [0.1–12.8]	4.1 (3.2) [0.1–12.8]	3.8 (2.8) [0.3–8.6]
Disease duration (Y), mean (SD) [range]	8.9 (4.8) [0.5–19.0]	9.1 (5.0) [2.4–19.0]	8.8 (4.6) [0.5–18.5]
Initial symptom			
Motor symptoms, n (%)	37 (66.1)	18 (60.0)	19 (73.1)
Dementia, n (%)	17 (30.4)	10 (33.3)	7 (26.9)
Autonomic failure, n (%)	2 (3.6)	2 (6.7)	0 (0)
Comorbid conditions			
Diabetes, n (%)	10 (17.9)	4 (13.3)	6 (23.1)
Hypertension, n (%)	23 (41.1)	13 (43.3)	10 (38.5)
Heart failure, n (%)	0 (0)	0 (0)	0 (0)
Ischemic heart disease prior to ¹²³ I-MIBG scintigraphy, n (%)	1 (1.8)	0 (0)	1 (3.9)
Renal failure [eGFR < 15 mL/min/1.73 m ²] or hemodialysis, n (%)	1 (1.8)	0 (0)	1 (3.9)
Peripheral neuropathy other than diabetic polyneuropathy, n (%)	0 (0)	0 (0)	0 (0)
Medication use at the time of ¹²³I-MIBG scintigraphy			
β-blocker other than labetalol, n (%)	5 (8.9)	1 (3.3)	4 (15.4)
Ca-channel blocker, n (%)	11 (19.6)	7 (23.3)	4 (15.4)

eGFR: estimated glomerular filtration rate; ¹²³I-MIBG: ¹²³I-meta-iodobenzyl-guanidine; LBD: Lewy body disease; SD: standard deviation; Y: years

Table 2: Diagnostic accuracy of ¹²³I-MIBG myocardial scintigraphy

¹²³ I-MIBG myocardial scintigraphy	Patients, n (%)				Diagnostic accuracy, % (95% CI)				Likelihood ratio (95% CI)	
	TP	FN	FP	TN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Early H/M ratio (Cutoff: 2.20)	21	9	1	25	70.0 (50.6–85.3)	96.2 (80.4–99.9)	95.5 (77.2–99.9)	73.5 (55.6–87.1)	18.2 (2.6–126.2)	0.31 (0.18–0.54)
Delayed H/M ratio (Cutoff: 2.20)	24	6	2	24	80.0 (61.4–92.3)	92.3 (74.9–99.1)	92.3 (74.9–99.1)	80.0 (61.4–92.3)	10.4 (2.7–39.9)	0.22 (0.11–0.45)
Delayed H/M ratio (Cutoff: 1.81)	24	6	0	26	80.0 (61.4–92.3)	100.0 (86.8–100.0)	100.0 (85.8–100.0)	81.2 (63.6–92.8)	-	0.20 (0.10–0.41)
Washout rate (Cutoff: 34%)	24	6	4	22	80.0 (61.4–92.3)	84.6 (65.1–95.6)	85.7 (67.3–96.0)	78.6 (59.0–91.7)	5.2 (2.1–13.0)	0.24 (0.11–0.49)
Any (Early H/M ratio or Delayed H/M ratio or Washout rate)	25	5	5	21	83.3 (65.3–94.4)	80.8 (60.6–93.4)	83.3 (65.3–94.4)	80.8 (60.6–93.4)	4.3 (1.9–9.7)	0.21 (0.09–0.47)
All (Early H/M ratio and Delayed H/M ratio and Washout rate)	20	10	0	26	66.7 (47.2–82.7)	100.0 (86.8–100.0)	100.0 (83.2–100.0)	72.2 (54.8–85.8)	-	0.33 (0.20–0.55)

CI: confidence interval; FN: false-negative; FP: false-positive; H/M ratio: heart/mediastinum ratio; ¹²³I-MIBG:

¹²³I-meta-iodobenzyl-guanidine; LR: likelihood ratio; NPV: negative predictive value; PPV: positive predictive value; TN: true-negative; TP: true-positive.

Figure Legends

Figure 1. Flow chart of participant selection.

BBAR: Brain Bank for Aging Research; H/M ratio: heart-to-mediastinum ratio; LBD: Lewy body disease; ^{123}I -MIBG: ^{123}I -meta-iodobenzyl-guanidine

Figure 2. Individual values for the heart-to-mediastinum (H/M) ratio and the washout rate of ^{123}I -meta-iodobenzyl-guanidine (^{123}I -MIBG) uptake in the pathologically diagnosed Lewy body disease (LBD) and non-LBD groups.

Early (A) and delayed (B) H/M ratios of ^{123}I -MIBG uptake show significant reductions in the LBD group. The washout rate (C) shows a significant increase in the LBD group. The box plot indicates the median, 25% and 75% quartiles with whiskers representing 1.5 times the interquartile range. The asterisk (*) indicates a p -value < 0.001. Early and delayed H/M ratios of ^{123}I -MIBG uptake (D) are shown for each of the following subgroups: motor symptoms, dementia, or autonomic failure, according to their initial complaints, further divided into 2 subgroups based on the time from symptom onset to scintigraphy (< 3 years or $\geq$ 3 years).

Figure 3. Receiver operating characteristic (ROC) curves for detecting Lewy body diseases based on the heart-to-mediastinum (H/M) ratios and washout rate.

ROC curves based on the early (A-Red, B) and delayed (A-Blue, C) H/M ratios and washout rate (A-Green, D). The bars indicate the 95% confidence interval at representative points. The asterisk (*) indicates the optimal cutoff point at which the sensitivity is maximized with the lower limit of the confidence interval for a specificity of 0.80 or higher. AUC: area under the ROC curve.

Figure 4. Correlation between the proportion of tyrosine hydroxylase (TH)-immunoreactive residual cardiac sympathetic fibers and cardiac ^{123}I -meta-iodobenzyl-guanidine (^{123}I -MIBG) uptake.

The scatter plots show the relationship between the proportion of residual TH-immunoreactive sympathetic fibers in the anterior wall of the left ventricle and the cardiac ^{123}I -MIBG uptake in the early (A) and delayed (B) H/M ratios. r : correlation coefficient.

Figure 5. Typical microscopic images of true-positive, false-positive, and false-negative cases of ^{123}I -meta-iodobenzyl-guanidine (^{123}I -MIBG) myocardial scintigraphy.

A true-positive case with Lewy body disease (LBD) and abnormal ^{123}I -MIBG myocardial scintigraphy presents with marked denervation of tyrosine hydroxylase (TH)-immunoreactive sympathetic fibers (A) and accumulation of α -synuclein (D) in the heart. Accumulation of α -synuclein is also identified in the sympathetic ganglion (G). A false-negative case with LBD and normal ^{123}I -MIBG myocardial scintigraphy presents with obscure denervation of TH-immunoreactive sympathetic fibers (B) and adequate accumulation of α -synuclein (E) in the heart. Only a small amount of α -synuclein accumulation is observed in the sympathetic ganglion (H). A false-positive case without LBD and with abnormal ^{123}I -MIBG myocardial scintigraphy presents with generally preserved TH-immunoreactive sympathetic fibers but sparse in some nerve fascicles (C), without accumulation of α -synuclein in the heart (F) or sympathetic ganglion (I). The counterstain is hematoxylin. Scale bar represents 50 μm (A–I).

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Autopsy Validation of the Diagnostic Accuracy of ¹²³I-Metaiodobenzylguanidine Myocardial Scintigraphy for Lewy Body Disease

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