Improving diagnostic accuracy of multiple system atrophy: A clinicopathological study of 203 cases

Yasuo Miki,[1,2] Sandrine C. Foti,[1] Yasmine T. Asi,[1] Eiki Tsushima,[4] Niall Quinn,[5] Helen Ling,[1,3*] and Janice L. Holton[1]

[1] Queen Square Brain Bank for Neurological Disorders, UCL Queen Square Institute of Neurology, 1 Wakefield Street, London WC1N 1PJ, UK

[2] Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan

[3] Reta Lila Weston Institute of Neurological Studies, UCL Queen Square Institute of Neurology, 1 Wakefield Street, London WC1N 1PJ, UK

[4] Department of Comprehensive Rehabilitation Science, Hirosaki University Graduate School of Health Sciences, Hirosaki 036-8564, Japan

[5] UCL Queen Square Institute of Neurology, London WC1N 3BG, UK

Correspondence to Dr. Helen Ling,

Queen Square Brain Bank for Neurological Disorders and Institute of Neurology, University College London

1 Wakefield Street London WC1N 1PJ, UK

Email: h.ling@ucl.ac.uk

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Abstract

Clinical diagnosis of multiple system atrophy (MSA) is challenging and many patients with Lewy body disease (LBD; ie, Parkinson's disease or dementia with Lewy bodies) or progressive supranuclear palsy (PSP) are misdiagnosed as having MSA in life. The clinical records of 203 patients with a clinical diagnosis of MSA were reviewed to identify diagnostic pitfalls. We also examined twelve features supporting a diagnosis of MSA (red flag features: orofacial dystonia, disproportionate antecollis, camptocormia and/or Pisa syndrome, contractures of hands or feet, inspiratory sighs, severe dysphonia, severe dysarthria, snoring, cold hands and feet, pathologic laughter and crying, jerky myoclonic postural/action tremor and polyminimyoclonus) and seven disability milestones (frequent falls, use of urinary catheters, wheelchair dependent, unintelligible speech, cognitive impairment, severe dysphagia, residential care). 160 out of 203 cases (78.8%) were correctly diagnosed in life and had pathologically confirmed MSA. The remaining 21.2% (43/203) had alternative pathological diagnoses including LBD (12.8%; N=26), PSP (6.4%; N=13), cerebrovascular diseases (1%; N=2), amyotrophic lateral sclerosis (0.5%; N=1) and cerebellar degeneration (0.5%; N=1). More MSA patients developed ataxia, stridor, dysphagia and falls than LBD patients, while resting tremor, pill-rolling tremor and hallucinations were more frequent in LBD. Although patients with MSA and PSP shared several symptoms and signs, ataxia and stridor were more common in MSA. Multiple logistic regression analysis revealed increased likelihood of MSA vs. LBD and PSP if a patient developed orthostatic hypotension or urinary incontinence with the requirement for urinary catheters (MSA vs. LBD: odds ratio (OR): 2.0, 95% confidence interval (CI): 1.1-3.7, P=0.021; MSA vs. PSP: OR: 11.2, 95% CI: 3.2-39.2, P<0.01). Furthermore, autonomic dysfunction within the first three years from onset can

differentiate MSA from PSP (MSA vs. PSP: OR: 3.4, 95% CI: 1.2-9.7, *P*=0.023). MSA patients with predominant parkinsonian signs (MSA-P) had a higher number of red flag features than patients with LBD (OR: 8.8, 95% CI: 3.2-24.2, *P*<0.01) and PSP (OR: 4.8, 95% CI: 1.7-13.6, *P*<0.01). The number of red flag features in MSA with predominant cerebellar signs (MSA-C) was also higher than in LBD (OR: 7.0, 95% CI: 2.5-19.5, *P*<0.01) and PSP (OR: 3.1, 95% CI: 1.1-8.9, *P*=0.032). MSA patients had shorter latency to reach use of urinary catheter and longer latency to residential care than PSP patients, whereas LBD patients took longer to reach multiple milestones than MSA patients. The present study has highlighted features which should improve the antemortem diagnostic accuracy of MSA.

Introduction

Multiple system atrophy (MSA) is an adult-onset, sporadic, rapidly progressing, fatal neurodegenerative disease that manifests with dysautonomia, plus cerebellar ataxia and/or poorly levodopa-responsive parkinsonism (Déjerine and Thomas, 1900; Shy and Drager 1960; Adams *et al*., 1961; Graham *et al*., 1969). Typically, patients with MSA die within nine years after its onset. Although certain medications can ease some of the symptoms for those affected (Wenning *et al*., 2013; Low *et al*., 2015), there is no cure. It is pathologically characterized by neuronal cell loss with the presence of glial cytoplasmic inclusions whose major component is phosphorylated α-synculein (Papp *et al.*, 1989; Wakabayashi *et al*., 1998). MSA is classified into two clinical subgroups, depending on the predominant motor presentation: a parkinsonian variant reflecting striatonigral degeneration (MSA-P) and a cerebellar variant related to olivopontocerebellar atrophy (MSA-C) (Fanciulli *et al*., 2015). According to the second consensus diagnostic criteria for MSA published in 2008, a "definite" diagnosis of MSA requires neuropathological confirmation, whereas a diagnosis of "probable" or "possible" MSA is made based on clinical grounds. In addition to these diagnostic categories, supporting (red flags) or non-supporting features are used for the clinical diagnosis of MSA (Gilman *et al*., 2008). Although the current diagnostic criteria are validated with high positive predictive values (PPVs) of "possible" and "probable" MSA (91% and 96%, respectively), a recent clinicopathological study has revealed that out of 134 patients clinically diagnosed with MSA meeting the diagnostic criteria for "probable" or "possible" MSA, only 83 (62%) had pathologically confirmed MSA. The remaining 51 cases (38%) had other conditions, including Lewy body disease (LBD), with a pathological diagnosis of either Parkinson's disease (PD n=8) or dementia with Lewy bodies (DLB n=19), progressive supranuclear

palsy (PSP n=15) and cerebrovascular disease (CVD n=2), that share some clinical features with MSA (Osaki *et al*., 2009; Koga *et al*., 2015). Thus, the diagnostic accuracy of a diagnosis of MSA using the current clinical criteria remains suboptimal.

Accumulating evidence has suggested that MSA cases have more heterogeneous clinical phenotypes than outlined in the current criteria. While poor levodopa response is considered a mandatory feature for "probable" MSA, and an additional feature for "possible" MSA, 30 to 50% of patients with MSA show a beneficial response to levodopa (Gilman *et al*., 2008; Wenning *et al*., 2013; Low *et al*., 2015). MSA can usually be distinguished from PD by a more rapid disease progression, poorer levodopa response, and the early presence of autonomic dysfunction (Gilman *et al*., 2008). Our group and others have reported pathologically confirmed MSA cases with atypical features deviating from the classic well delineated clinical phenotypes of MSA-P and MSA-C. These subgroups may be referred to as MSA variants. We reported four MSA cases with prolonged disease duration of 15 years or more, who had late onset of autonomic dysfunction and levodopa-induced dyskinesia (Petrovic *et al*., 2012). Although vertical gaze palsy in MSA is mild compared with PSP and upward gaze is more affected than down gaze, 26.7% of patients with probable MSA-P had vertical gaze palsy (Anderson *et al*., 2008; Höglinger *et al*., 2017). Cognitive impairment, which is described as a non-supporting feature for MSA, was reported in up to 32% of patients with MSA (Wenning *et al*., 1997; Brown *et al*., 2010; Cykowski *et al*., 2015; Koga *et al*., 2017). Aoki et al. reported four patients with pathologically proven MSA who developed frontotemporal dementia without autonomic dysfunction (Aoki *et al*., 2015). Riku et al., investigating 161 consecutive patients with MSA, found four cases who died with autonomic dysfunction in the absence of motor symptoms (Riku *et al*., 2017). The extent to which

red flags are useful for the differentiation between MSA and MSA lookalikes such as PD, PSP and CVD remains unknown: Firstly, the validity of red flag features in supporting the clinical diagnosis of MSA has yet to be fully evaluated using autopsy proven MSA cases; secondly, they are designed to principally differentiate between MSA-P and PD, but not other MSA lookalikes including PSP; and finally, there has been no study on red flags for MSA-C (Gilman *et al*., 2008; Köllensperger *et al*., 2008).

In the present study, we retrospectively examined the clinical and pathological records of 203 patients with clinically diagnosed MSA. Their clinical manifestations were compared and contrasted according to their pathological diagnoses. We aimed to improve antemortem diagnostic acumen by identifying diagnostic pitfalls in patients with PD or PSP who were sufficiently atypical to have been misdiagnosed as MSA. We also examined the clinical utility of individual red flags to differentiate MSA from MSA lookalikes that had non-MSA pathology at post-mortem.

Materials and Methods

Patients

217 patients with a final clinical diagnosis of MSA were identified from the archive of 2157 cases referred to the Queen Square Brain Bank for Neurological Disorders between 1989 and 2017. Consent for brain donation was obtained from the patients prior to death and/or consent for post-mortem examination was obtained from the next of kin after death. The brain donation program and protocols have received ethical approval for donation and research by the NRES Committee London – Central and tissue is stored for research under a license issued by the Human Tissue Authority (No. 12198).

Medical record review

We systematically reviewed all available medical records for 217 cases who died with a clinical diagnosis of MSA. This including the primary care medical records, correspondence between medical specialists and general practitioners, National Hospital for Neurology and Neurosurgery medical files, and the Queen Square Brain Bank self-assessment data. All patients had been assessed by experienced hospital specialists (consultant physicians, geriatricians, general neurologists, movement disorders specialists) during the course of their illness. The information from the case notes was extracted by one neurologist (Y. M.) who was blinded to the pathological diagnosis. 14 cases were excluded from the 217 cases: six patients had inadequate medical records regarding disease progression, seven patients had severe autonomic neuropathy due to other causes including diabetic autonomic neuropathy, and one patient had a genetic mutation of microtubule-associated protein tau. The flow chart of the study design is shown in Fig.1.

Many neurological signs and symptoms were selected for evaluation. They included clinical features for "probable" and "possible" MSA, red flags and non-supporting features in the current diagnostic criteria for MSA (Gilman *et al*., 2008). In comparing PSP with MSA, several core features for "probable" PSP were also included in the present study (Höglinger *et al*., 2017). These are: i) age of onset: age, in years, when the first motor symptom considered to be attributable to the neurological disorder was reported; ii) time to final clinical diagnosis: time between the age of onset and the age when the last diagnosis recorded before death was mentioned; iii) disease duration: time between the age of onset and the age at death; iv) duration span of examinations by

hospital specialists; v) latency between last examinations and death; vi) family history: recorded as present if a first- or second-degree family history of parkinsonism or ataxia was documented; vii) levodopa: the maximum dose of levodopa during the course of illness; viii) beneficial levodopa response: moderate to good response described by a clinician; ix) bradykinesia; x) rigidity; xi) resting tremor; xii) typical pill-rolling tremor, xiii) postural/action tremor; xiv) intention tremor; xv) positive pull test: recorded as positive if a patient was unable to maintain his/her stability and would fall backwards if not caught after an examiner delivered a quick backwards pull; xvi) early positive pull test within three years of onset; xvii) falls: recorded as present if unprovoked falls were mentioned; xviii) early falls within three years of onset; xix) gait freezing ; xx) early gait freezing within three years of onset; xxi) ataxia: recorded as present if gait ataxia, cerebellar dysarthria or limb ataxia was noted; xxii) early ataxia within three years of onset; xxiii) hyperreflexia with Babinski sign; xxiv) stridor; xxv) dysphagia within five years of onset; xxvi) vertical gaze palsy: recorded as present if upward or downward vertical gaze palsy was mentioned by a clinician; xxvii) frontal release signs: defined as presence of at least one of the following signs: Gegenhalten, snout reflex, palmomental reflex or grasp reflex; xxviii) impairment of frontal lobe function, defined as presence of at least one of the following symptoms: personality change, executive dysfunction, disinhibition, or stereotypy; xxix) depression; xxx) hallucination; xxxi) REM sleep behavior disorder: recorded as present if confirmed on polysomnography or if it was clinically suspected based on behavioral description by the bed partner; xxxii) urinary urgency; xxxiii) urinary frequency; xxxiv) incomplete bladder emptying; xxxv) urinary incontinence; xxxvi) orthostatic hypotension: divided into two subcategories (severe or mild orthostatic hypotension). Severe orthostatic hypotension was defined as a > 30 mm

Hg systolic or 15 mm Hg diastolic blood pressure drop on standing, or repeated episodes of syncope. Mild orthostatic hypotension was defined as a significant (20/10) drop that did not meet the above 30/15 drop; xxxvii) red flags: warning signs that raise the clinical suspicion of MSA. They consist of clinical features including orofacial dystonia, disproportionate antecollis, camptocormia and/or Pisa syndrome, contractures of hands or feet, inspiratory sighs, severe dysphonia, severe dysarthria, snoring, cold hands and feet, pathologic laughter and crying, jerky myoclonic postural/action tremor and polyminimyoclonus (Gilman *et al*., 2008; Köllensperger *et al*., 2008). Because polyminimyoclonus is now considered to be specific to MSA, it was included in the red flags in this study (Okuma *et al*., 2005). We also analyzed the severity of autonomic dysfunction and the number of red flags to predict the likelihood of MSA. In addition, seven milestones of disease progression were assessed as previously reported (O'Sullivan *et al*., 2008). These were frequent falls (falls occurring more than twice a year or the documentation of 'frequent' or 'regular' falls); cognitive impairment (a documentation by a clinician regarding cognitive symptoms including impaired short-term memory, executive dysfunction, disorientation or personality change); unintelligible speech or the requirement for communication aids (a description in the clinical records regarding persistent incomprehensible speech or the use of communication aids due to severe dysarthria or dysphonia) or severe dysphagia or requirement for percutaneous endoscopic gastrostomy (PEG) tube placement for feeding (a description in the clinical records that patients were persistently unable to swallow or dependent on a PEG tube for their nutrition intake because of their severe dysphagia); dependence on a wheelchair (a documentation concerning inability to mobilise without a wheelchair due to parkinsonism or ataxia); use of urinary catheters (the persistent requirement for urinary catheters due to

severe neurogenic bladder dysfunction); placement in residential or nursing home care. All clinical signs or symptoms were recorded as unknown if they were not specifically mentioned.

Neuropathological methods

All cases were selected from the Queen Square Brain Bank, where brain donation took place according to ethically approved protocols, and the tissues were stored under a license from the Human Tissue Authority. The brains were fixed with 10% buffered formalin for three weeks. Eight-µm-thick, formalin-fixed, paraffin-embedded sections were cut from multiple regions throughout the brain. Sections were first stained with hematoxylin and eosin. They were then subjected to immunohistochemical processing with the avidin-biotin-peroxidase complex method with diaminobenzidine as the chromogen. For routine histological investigations, mouse monoclonal antibodies against amyloid β (M0872; Dako, Ely, UK; 1:100), α-synuclein (MA1-90342; Thermo Scientific, Waltham, MA; 1:1,500), tau (MN1020; Thermo Scientific; 1:600) and transactivation response DNA-binding protein 43kDa (TDP-43) (H00023435-M01; Abnova, Taipei, Taiwan; 1:6,000) and a rabbit polyclonal antibody against ubiquitin (Z0458; Dako; 1:200) were used. Sections in some cases were also stained with the Bielschowsky silver impregnation for the assessments of neuritic plaques and neurofibrillary tangles (NFTs). Based on the degree of neuronal cell loss and glial cytoplasmic inclusions, MSA was subclassified into MSA of striatonigral degeneration predominant type (MSA-SND), MSA of olivopontocerebellar predominant type (MSA-OPCA) and MSA with equal involvement of SND and OPCA (MSA-SND=OPCA) (Ozawa *et al*., 2004). Lewy body type (brainstem predominant, limbic and diffuse neocortical) was assigned based upon

pattern of Lewy-related pathology according to the consensus criteria for pathologic assessment of dementia with Lewy bodies (McKeith *et al*., 2017). It is difficult to immunohistochemically distinguish between concomitant Lewy bodies in MSA and neuronal cytoplasmic inclusions, another pathological hallmark of MSA (Papp *et al*., 1992). Therefore, sections stained with hematoxylin and eosin were used for the assessment of concomitant Lewy bodies in the substantia nigra and the locus coeruleus. Neuritic plaques and NFTs were also evaluated according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scheme and Braak NFT stage, respectively (Alafuzoff *et al*., 2008; Hyman *et al*., 2012).

Statistical analysis

All statistical analyses in the present study were performed using SPSS 25.0 (SPSS Inc,USA) or R commander (Rcmdr 1.3-5 package, R2.8.1). Pathologic groups consisting of more than three cases (MSA, PD and PSP) were included for statistical analyses. The Chi-square or Fisher's exact tests with Bonferroni correction was performed for categorical variables. After Shapiro-Wilk test, one-way ANOVA followed by Tukey test or Kruskal-Wallis test followed by Steel-Dwass test was used for continuous variables. We also performed multiple logistic regression analysis to estimate the association between MSA and MSA lookalikes in CERAD plaque scheme, Braak NFT stage, autonomic dysfunction score or red flag score. Because accumulation of tau and amyloid β is influenced by the aging process, we adjusted for age in these evaluations. Odds ratio (OR) and 95% confidence interval (CI) were used as the main effect size. In addition, Kaplan-Meier curves were generated to compare latencies to reach clinical milestones between MSA and MSA lookalikes. Log Rank (Mantel-Cox) with Bonferroni

correction was used for comparison of two groups. A probability value of less than 0.05 (P<0.05) was considered to be significant.

Data availability

The raw data that support the findings of the present study are available on request from the corresponding author.

Results

Demographic characteristics of patients with MSA and MSA lookalikes

Table1 shows the demographic data of patients with MSA, and MSA lookalikes who were clinically misdiagnosed as having MSA, but had another diagnosis at post-mortem. Of the 203 patients with a clinical diagnosis of MSA, 160 patients had MSA at post-mortem examination (78.8 %). The pathological diagnoses in the other 43 cases were LBD (n = 26, 12.8%), PSP (n = 13, 6.4%), CVD (n = 2, 1%), amyotrophic lateral sclerosis (n = 1, 0.5%) and cerebellar degeneration (n = 1, 0.5%). Diagnostic acumen did not show significant improvement between 1989 and 2017 despite two new sets of consensus diagnostic criteria in 1998 and 2008 (Gilman *et al*., 1999; Gilman *et al*., 2008) (Supplementary Fig. 1). The vast majority (96.6%) of the patients were reviewed at least once by neurologists during the course of their illness. Significant difference was found in the proportion of neurologists vs. non-specialists who made a diagnosis between MSA and MSA lookalikes subgroups, suggesting the better diagnostic accuracy by neurologists (96.9% and 85.3%, respectively, P=0.021). The age at onset and death in patients with MSA was 56.7 ± 8.4 years (range 34-78) and 63.7 ± 7.8 years (range 39-83), respectively, both of which were significantly younger than those of LBD and PSP patients. Time from

disease onset to final clinical diagnosis in MSA was significantly shorter than that in LBD (MSA vs. LBD: 3.7 ± 2.3 vs. 6.6 ± 4.4 years, $P<0.01$). Duration from disease onset to death in MSA was also significantly shorter compared with that in LBD (MSA vs. LBD: 7.0 ± 3.1 vs. 10.7 ± 5.0 years, $P<0.01$). MSA patients had shorter duration span of examination by hospital specialists compared with patients with LBD, which is in keeping with the shorter disease duration of MSA. There was no difference in gender, latency between last examinations and death, presence of a first- or second-degree family history of parkinsonian disorders or ataxia, levodopa dose and beneficial levodopa response between MSA and MSA lookalikes. All cases underwent retrospective assessment of clinical features. This showed that 70 patients met diagnostic criteria for "probable", and 100 patients for "possible", MSA. The remaining 33 cases lacked aspects of the clinical information required for the current diagnostic criteria. The positive predictive values of "probable" MSA and "possible" MSA were 91.4% and 75%, respectively.

The brain weights did not differ between MSA, LBD and PSP. The breakdown of MSA pathologic subtypes was as follows: MSA-SND, 28.8%; MSA-OPCA, 35.6%; MSA-SND=OPCA, 35.6%. Examination of concomitant neuropathology did not show significant difference in Lewy-related pathology between MSA and PSP. Senile plaques and NFTs were similarly found between MSA and MSA lookalike subgroups. MSA-SND, MSA-OPCA and MSA-SND=OPCA had similar stages of senile plaques and NFTs.

Clinical manifestations of MSA and MSA lookalikes

Table2 shows the frequencies of clinical features between patients with MSA and MSA lookalikes. Patients with MSA, LBD and PSP similarly presented with some parkinsonism. However, compared with patients with MSA, more LBD patients showed

resting tremor (MSA vs. LBD: 27.5% vs. 53.8%, P=0.021), typical pill-rolling tremor (MSA vs. LBD: 3.7% vs. 26.9%, P<0.01), and hallucination (MSA vs. LBD: 5% vs. 34.6%, P<0.01). On the other hand, patients with MSA more frequently developed ataxia (MSA vs. LBD: 62.5% vs. 3.8%, P<0.01), early ataxia within three years of onset (MSA vs. LBD: 36.8% vs. 0%, P<0.01), stridor (MSA vs. LBD: 31.8% vs. 7.6%, P=0.34), dysphagia within five years of onset (MSA vs. LBD: 46.2% vs. 15.3%, P<0.01), falls (MSA vs. LBD: 82.5% vs. 61.5%, P=0.042) and early falls within three years of onset (MSA vs. LBD: 42.5% vs. 15.3%, P=0.025). The frequencies of clinical features were also compared between MSA and PSP. More patients with MSA had ataxia (MSA vs. PSP: 62.5% vs. 23.0%, P=0.015) and stridor (MSA vs. PSP: 31.8% vs. 0%, P=0.034) than patients with PSP. Vertical gaze palsy (up *or* down) was more common in PSP (MSA vs. PSP: 20.6% vs. 61.5%, P<0.01). Impaired *down gaze* was found in 21.2% of MSA patients with vertical gaze palsy (7/33) and 37.5% of PSP patients with vertical gaze palsy (3/8). No difference was seen in the frequency of frontal cognitive or behavioral presentation between MSA, LBD and PSP. The LBD and PSP patients are not representative of typical LBD and PSP because our study highlights atypical presentations of LBD and PSP.

Autonomic dysfunction in MSA and MSA lookalikes.

We compared the frequencies of autonomic dysfunction between MSA and MSA lookalikes (Table3). More than 84% of the patients among the groups had various degrees of autonomic failure. We then evaluated the features of autonomic failure for a diagnosis of “possible” MSA. There was no difference in the frequency of urinary urgency, frequency or incomplete bladder emptying, or mild orthostatic hypotension

between MSA and MSA lookalikes. Next, we examined the frequencies of urinary incontinence and severe orthostatic hypotension, which are listed in the diagnostic criteria for "probable" MSA (Gilman *et al*., 2008). Compared with patients with PSP, more MSA patients presented with urinary incontinence (MSA vs. PSP: 78.1% vs. 30.8%, $P<0.01$) and severe orthostatic hypotension (MSA vs. PSP: 58.8% vs. 15.4%, $P<0.01$). We also performed multiple logistic regression analysis to estimate how the combination of autonomic dysfunction (severe orthostatic hypotension and/or urinary incontinence) can influence the likelihood of MSA. This revealed increased likelihood having MSA than PSP if a patient developed severe hypotension and/or urinary incontinence (OR: 8.1, 95% CI: 2.9–22.4; $P<0.01$). As with MSA patients, typical LBD patients can present with moderate to severe autonomic dysfunction (Wenning et al., 1999; Kaufmann *et al*., 2004). Thus, we examined the more restrictive combination of autonomic dysfunction (severe orthostatic hypotension and/or urinary incontinence with use of urinary catheters) to discriminate between MSA and atypical LBD mistaken for MSA-P. The OR of the strict combination was 2.0 between MSA and LBD (95% CI: 1.1–3.7; $P=0.021$) and 11.2 between MSA and PSP (95% CI: 3.2–39.2; $P<0.01$). 38.1% of MSA patients (61/160) developed both features, whereas only 7.7% of LBD patients (2/26) and no PSP patients had both features (Supplementary Fig.2). These findings indicate that the combination of severe orthostatic hypotension and/or urinary incontinence with the requirement of urinary catheters can be useful to differentiate between MSA and MSA lookalikes.

Red flag features in MSA and MSA lookalikes

Red flags were first introduced to differentiate MSA-P from PD, but not from

PSP. Little is known about red flags in the differential diagnosis between MSA-C and MSA lookalikes (Quinn. 1989; Gilman *et al*., 2008; Köllensperger *et al*., 2008). We investigated the presence of these established red flags in the MSA-P, MSA-C, LBD and PSP groups (Table4). Compared with LBD patients, patients with MSA-P and MSA-C more frequently developed severe dysarthria (MSA-P vs. LBD: 43.7% vs. 3.8%, $P<0.01$; MSA-C vs. LBD: 49.1% vs. 3.8%, $P<0.01$) and pathologic laughter or crying (MSA-P vs. LBD: 22.3% vs. 0%, $P=0.012$; MSA-C vs. LBD: 24.6% vs. 0%, $P=0.011$). Significant difference was also found in the frequency of snoring between MSA-C and PSP (MSA-C vs. PSP: 38.5% vs. 0%, $P=0.019$). Orofacial dystonia, inspiratory sighs, contractures of hands or feet, and polyminimyoclonus were found at more than twice the frequency in MSA-P compared with LBD and PSP, but this difference was not significant because of small numbers. Polyminimyoclonus was also reported in 10.5% of patients with MSA-C but none with LBD or PSP. Patients with MSA, LBD and PSP similarly developed disproportionate antecollis. Next, we performed multiple logistic regression analysis to establish whether the presence of a higher number of red flags increases the likelihood of an MSA diagnosis. No single red flag feature was useful to differentiate between MSA and MSA lookalikes including LBD or PSP. Then, red flag features that are present in at least 10% of MSA cases and less than 10% of lookalikes were selected to be included for this part of the analysis to ensure our findings are applicable as useful clinical pointers to differentiate MSA from LBD or PSP. The red flag features selected for LBD vs. MSA were orofacial dystonia, inspiratory sighs, contractures of hands or feet, polyminimyoclonus, severe dysarthria, pathologic laughter or crying, and cold hands and feet, whereas the features selected for PSP vs. MSA orofacial dystonia, inspiratory sighs, contractures of hands or feet, jerky myoclonic postural/action tremor, polyminimyoclonus,

severe dysphonia, and snoring. Patients with MSA-P had more red flags than patients with LBD (MSA-P vs. LBD: 1.5 ± 1.3 vs. 0.2 ± 0.5, OR: 8.8, 95% CI: 3.2–24.2, $P<0.01$) and PSP (MSA-P vs. PSP: 1.4 ± 1.3 vs. 0.3 ± 0.5, OR: 4.8, 95% CI: 1.7–13.6, $P<0.01$). The likelihood that a patient has MSA-P as opposed to LBD or PSP if the patient has any of the seven selected red flags (PPV) was 96.2% or 94.9%, respectively. Furthermore, the number of red flags in MSA-C was significantly higher than in PD (MSA-C vs. LBD: 1.2 ± 0.9 vs. 0.2 ± 0.5, OR: 7.0, 95% CI: 2.5–19.5, $P<0.01$) and PSP (MSA-C vs. PSP: 0.9 ± 0.9 vs. 0.3 ± 0.5, OR: 3.1, 95% CI: 1.1–8.9, $P=0.032$). The PPV of MSA-C versus LBD or PSP was 86% or 90.5%, respectively.

Early distinction of MSA from MSA lookalikes

We further examined the frequencies of autonomic dysfunction or red flags within the first three years from onset for early distinction of MSA from MSA lookalikes. Compared with PSP patients, MSA patients more frequently developed autonomic dysfunction including urinary urgency, frequency, or incontinence, incomplete bladder emptying, or mild or severe orthostatic hypotension, which is required for a diagnosis of "probable" or "possible" MSA in the current diagnostic criteria (MSA vs. PSP: 58.8% vs. 15.3%, $P<0.01$) (Gilman *et al*., 2008). Furthermore, multiple logistic regression analysis confirmed an increased likelihood of MSA vs. PSP if a patient developed any of the autonomic dysfunctions described above (MSA vs. PSP: OR: 3.4, 95% CI: 1.2-9.7, $P=0.023$) (Table5). There were no differences in the frequencies of early red flag features between MSA and MSA lookalikes (data not shown). The number of positive red flag features is too small for multiple logistic regression analysis to be performed.

Clinical milestones in pathologically diagnosed MSA and MSA lookalikes

Finally, we studied disability milestones relevant to MSA and MSA lookalikes (Table6). More patients with MSA than patients with LBD and PSP required use of urinary catheters (MSA vs. LBD: 75% vs. 34.6%, $P<0.01$; MSA vs. PSP: 75% vs 23.1%, $P<0.01$). Wheelchair dependence (MSA vs. LBD: 53.1% vs. 23.0%, $P=0.013$) and unintelligible speech (MSA vs. LBD: 50% vs. 15.3%, $P<0.01$) were seen more in MSA than LBD, while cognitive impairment occurred more in patients with LBD than patients with MSA (MSA vs. LBD: 20% vs. 46.2%, $P=0.032$). 38.5% of patients with PSP had dementia. In addition, multiple comparisons showed that MSA patients reached frequent falls (MSA vs. LBD: 3.4 ± 2.3 vs. 6.1 ± 4.0 years, $P=0.037$), unintelligible speech (MSA vs. LBD: 6.2 ± 2.5 vs. 13.3 ± 5.6 years, $P=0.018$), and cognitive impairment (MSA vs. LBD: 4.1 ± 2.8 vs. 8.8 ± 5.0 years, $P=0.015$) earlier in their disease course than LBD patients (Table6). Kaplan-Meier curves also confirmed that compared with patients with LBD, MSA patients had shorter latencies to reach frequent falls ($P=0.043$), use of urinary catheter ($P<0.01$), wheelchair dependence ($P<0.01$) and unintelligible speech ($P<0.01$). On the other hand, MSA patients had shorter latency to reach use of urinary catheter ($P<0.01$) and longer latency to residential care ($P<0.01$) than patients with PSP. There was no difference in time to reach five disability milestones (frequent falls, wheelchair dependence, cognitive impairment, unintelligible speech and severe dysphagia) when comparing MSA and PSP (Fig.2).

Discussion

In the present study, the diagnostic accuracy of MSA was 78.8% (160/203), suggesting that antemortem diagnostic acumen remains a challenge. When patients

received a clinical diagnosis of "probable" MSA, 91.4% of patients had a correct pathological diagnosis. On the other hand, only 75% of patients with "possible" MSA were correctly diagnosed using these criteria. Out of the 43 clinically misdiagnosed patients, 95.3% (41/43) had a final clinical diagnosis of MSA-P, while 96.6% of patients with clinically diagnosed MSA-C (57/59) had a correct pathological diagnosis. 95.1% of the misdiagnosed MSA-P cases (39/41) showed varying degrees of autonomic dysfunction in addition to parkinsonism. Autonomic dysfunction in PD and PSP-parkinsonism (PSP-P), a clinical subtype of PSP frequently mistaken for MSA-P, is less common and severe than in MSA (Magalhães *et al*., 1995; O'Sullivan *et al.,* 2008; Williams *et al*., 2010; Oliveira *et al*., 2018). Thus, the presence and severity of autonomic dysfunction in these atypical parkinsonian disorders can predispose clinicians towards a misdiagnosis of MSA.

We have highlighted several clinical differences between definite MSA and atypical PD or PSP clinically mistaken for MSA-P. More MSA patients developed dysphagia, stridor and falls than our atypical LBD patients. Although patients with MSA and PSP shared many symptoms and signs, ataxia and stridor were more common in MSA. Multiple logistic regression analysis demonstrated that despite frequent development of autonomic dysfunction in our atypical LBD and PSP cases, LBD or PSP can be distinguished from MSA by the absence in LBD or PSP of severe orthostatic hypotension or urinary incontinence with use of urinary catheters (Table3). Furthermore, evaluating early autonomic dysfunction within the first three years from onset can be useful for early distinction of MSA from PSP (Table5). In the presence of a combination of seven red flag features including orofacial dystonia, inspiratory sighs, contractures of hands or feet, polyminimyoclonus, severe dysarthria, pathologic laughter or crying, and cold hands and

feet, a patient with one of these red flag features was 8.8 times more likely to have MSA than atypical LBD. If a patient developed one of these features including orofacial dystonia, inspiratory sighs, contractures of hands or feet, jerky myoclonic postural/action tremor, polyminimyoclonus, severe dysphonia, and snoring, the patient was 4.8 times more likely to have MSA than PSP (Table4). Taken together, our results clearly indicate that clinical establishment of autonomic dysfunction and the seven selected red flag features outlined above should improve antemortem diagnostic accuracy of MSA. Because our study is designed to distinguish between MSA and atypical PD or PSP clinically mistaken for MSA-P, the evaluation of autonomic dysfunction or red flag features would even more efficiently discriminate MSA from typical PD or PSP.

PD patients can sometimes mimic MSA-P especially when they develop moderate to severe autonomic dysfunction. The investigation of clinical features and disability milestones in the present study further revealed several characteristics in LBD with or without atypical features. Disease duration in our atypical LBD was shorter than those previously reported (10.7 vs. 12.8-15.8 years) (Hughes, et al., 1992; Kempster *et al*., 2010; Williams *et al*., 2010). However, the appearance of multiple clinical milestones in our LBD patients occurred only in the advanced disease stage and was still compatible with idiopathic PD. Typical pill-rolling tremor was strikingly less common in MSA (3.7%) than in LBD or PSP. Notably, the occurrence of documented pill-rolling tremor in 27% of the LBD cases misdiagnosed as MSA-P was only one third of the 75% typically seen in LBD, suggesting that lack of resting tremor can be a reason for its misdiagnosis as MSA and emphasizing that cases of LBD misdiagnosed as MSA are atypical (Hoehn and Yahr, 1967; Hughes *et al*., 1993). Hallucinations were least (5%) frequent in our MSA cases, versus 34.6% in LBD cases and 15.4% in PSP cases. This, together with greater

cognitive problems, may explain why, despite having more motor impairment, many fewer cases of MSA than LBD ended up in residential care. On the other hand, the frequency of visual hallucination in idiopathic PD cases is reported to be 61.2% (Kempster *et al*. 2010), so the relative infrequency of hallucinations may have favoured misdiagnosis of LBD cases as MSA-P. Dementia is a common clinical manifestation in idiopathic PD with a cumulative incidence approaching 80% (Green et al., 2002; Aarsland and Kurz, 2010). In the present study, 46% of patients with LBD developed dementia during the disease course. Again, cases lacking or less frequently developing some of these representative manifestations of PD may favour misdiagnosis as MSA-P.

PSP-P can also be mistaken for MSA-P inasmuch as these disorders share some features such as akinetic rigid type parkinsonism with poor response to levodopa. In addition, some patients with PSP have even presented with resting or jerky postural/action tremor (Williams *et al.,* 2009). Previous studies have used clinical milestones to compare the rates of disease progression between MSA and PSP-P (O'Sullivan *et al.,* 2008; Williams *et al*., 2010; Oliveira *et al*., 2018). Our misdiagnosed PSP cases had a shorter disease duration compared with typical PSP-P cases reported in these studies (7.9 vs 9.0-12.7 years) highlighting that they represent an atypical PSP group. Despite variations in clinical milestones between these studies, our atypical PSP cases reached wheelchair dependence at least 2.2 years earlier than those in the above papers. Autonomic dysfunction in typical PSP-P is not as frequent and severe as in MSA (O'Sullivan *et al.,* 2008; Williams *et al*., 2010; Oliveira *et al*., 2018). In the present study, 84.6% of our atypical PSP cases developed some impairments of autonomic function. Either urinary incontinence or severe orthostatic hypotension is required for a diagnosis of "probable" MSA. Urinary incontinence was found in 30.8% of our atypical PSP cases. 15.4% of

patients with PSP experienced severe orthostatic hypotension, a drop of more than 30 mmHg systolic or 15 mmHg diastolic on standing or repeated episodes of syncope. Despite the absence of comparative studies between typical and atypical PSP-P mistaken for MSA-P, PSP-P with autonomic dysfunction and more rapidly progressive clinical course is more likely to be misdiagnosed as MSA-P. Patients with LBD and PSP more frequently required residential care in their advanced stage compared with MSA patients (Table 6). Patients with dementia and hallucinations often need residential or nursing home care as disease progresses (Goetz and Stebbings, 1993). In the present study, 38.5 to 46.2% of patients with LBD and PSP had dementia, while the frequency of cognitive impairment in MSA was 20%. The high frequencies of cognitive impairment in patients with atypical LBD and PSP may contribute to an increased requirement for residential care.

MSA and LBD, both of which are classified as synucleinopathies, have different characteristics of concurrent pathologies. Robinson et al. reported that the incidence of amyloid β or tau co-pathology increased along with the propagation of α-synuclein in LBD brains. In contrast, patients with MSA had a similar burden of amyloid β or tau to that in age-matched controls (Robinson *et al*., 2018). In the present study, the severity of amyloid β or tau co-pathology did not differ between MSA-SND, -OPCA and -SND=OPCA pathological subgroups, indicating that the propagation of α-synuclein in MSA brains might not accelerate the accumulation of concomitant amyloid β or tau. Misfolded proteins can play a role in proteopathic seeding, causing further aggregation of abnormal proteins (Clinton *et al*., 2010). However, there have been no reports that α-synuclein in glial cytoplasmic inclusions can synergistically interact to give rise to concomitant Lewy bodies in MSA. In the present study, concomitant Lewy-related

pathology was present in 7.5% of patients with MSA (12/160). 12% of healthy individuals older than 60 years of age is known to have incidental Lewy bodies (Klos *et al*., 2006). Thus, concomitant Lewy-related pathology in our MSA cases is likely to be simply age-related. Pathologic analysis in the present study showed no difference in co-pathology including α-synuclein, amyloid β and tau, between MSA and MSA lookalikes. Therefore, co-pathologies may not influence the atypical presentations in MSA lookalikes.

Lewy-related pathology was found in 23.1% of our patients with PSP (3/13). Indeed, concomitant Lewy-related pathology is reported in up to 31 % of PSP cases (Mori *et al*., 2002; Uchikado *et al*., 2006; Robinson *et al*., 2018). The incidence of concomitant protein aggregate pathologies differs even among disorders that belong to the same proteinopathy. Among tauopathies, PSP had more concurrent α-synuclein compared with other tauopathies including corticobasal degeneration or Pick's disease. Up to 22% of patients with LBD had concomitant TDP-43, whereas concomitant TDP-43 was very rare in MSA (Koga *et al*., 2018; Robinson *et al*., 2018). These findings suggest that other unknown factors are required to trigger concomitant protein aggregation in addition to proteopathic seeding. Further study is essential to investigate this question.

There are limitations to the present study. It is the inherent limitation of all brain bank based retrospective clinicopathological studies that, although 96.6% of the patients were reviewed at least once by neurologists during the course of their illness, clinical symptoms/signs and other clinical information including levodopa response was not necessarily documented in every case at every follow up appointment. In fact, cognitive and language functions were not, until recently, consistently evaluated, leading to cognitive impairment or psychiatric symptoms being underestimated especially in historically older cases. In addition, the neurological signs/symptoms that were selected

for evaluation could have occurred at any time during the disease course. Therefore, the frequencies of symptoms in the different groups as reported in this study can reflect the cumulative effect of disease duration. The selection bias of brain bank postmortem cases may lead to different results compared with previous clinical studies. Indeed, our study had better diagnostic accuracy than the Koga study (Koga *et al*., 2015). Our brain bank may have more cases that have come through specialist clinics, whereas many of the patients in the Koga study were derived from the community setting. Thus, the diagnostic yield for MSA might potentially be affected because of the difference in the source of cases in the brain banks. Because our brain bank specializes in parkinsonian disorders, patients with idiopathic late onset cerebellar ataxia, distinguished from MSA-C by the absence of prominent autonomic symptoms, are not recruited unless they are clinically diagnosed as having MSA. In the present study, there is one patient with the clinical diagnosis of MSA due to the presence of severe orthostatic hypotension and urinary incontinence, but neuropathological examination showed cerebellar degeneration of unknown cause with negative genetic testing for common spinocerebellar ataxia (SCA) mutations (SCA1, 2, 3, 6, 7, 12 and 17). The predilection for atypical parkinsonian cases referred to our brain bank donor scheme may be one of the reasons for the higher proportion of atypical MSA-P cases and lower proportion of idiopathic late onset cerebellar ataxia in the present cohort. Due to the nature of our study design, there was considerable difference in sample sizes among disease groups and therefore measures of statistical significance with robust correction for multiple comparisons may have obscured real differences.

We have detailed clinical and pathological data based on the largest sample size of MSA postmortem cases. Our study has demonstrated how evaluating autonomic

dysfunction and red flags can contribute to differentiation between MSA and MSA lookalikes. This should help improve *in vivo* diagnosis of MSA and enable appropriate recruitment into future clinical studies.

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Competing interests

None.

Figure legends

Fig. 1

Study design. Abbreviations: MSA = multiple system atrophy; LBD = Lewy body disease; PSP = progressive supranuclear palsy: CVD = cerebrovascular disease; ALS = amyotrophic lateral sclerosis.

Fig. 2

Kaplan-Meier curves illustrating latencies of clinical milestones between MSA and MSA lookalikes. (A) Latencies to reach frequent falls between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P=0.043; MSA vs PSP: df = 1, P=1 (B) Latencies to reach use of urinary catheter between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P<0.01; MSA vs. PSP: df = 1, P<0.01. (C) Latencies to reach wheelchair dependence between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel Cox) with Bonferroni correction, df = 1, P<0.01; MSA vs. PSP: df = 1, P=1. (D) Latencies to reach unintelligible speech between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P<0.01; MSA vs. PSP: df = 1, P=1. (E) Latencies to reach cognitive impairment between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P=1; MSA vs. PSP: df = 1, P=0.65 (F) Latencies to reach severe dysphagia between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P=0.062; MSA vs. PSP: df = 1, P=1. (G) Latencies to reach residential care between MSA, LBD and PSP (years). MSA vs. LBD: Log Rank (Mantel-Cox) with Bonferroni correction, df = 1, P=0.34; MSA vs. PSP: df = 1, P<0.01. Blue line: MSA; red line: LBD;

green line: PSP.

Supplementary Fig. 1

The proportion of cases misdiagnosed as having MSA for every 5 years. Numerators indicate the number of misdiagnosed cases as having MSA. Denominators indicate the number of cases with a final clinical diagnosis of MSA.

Supplementary Fig. 2

The proportion of patients who needed the restrictive combination of autonomic dysfunction (severe orthostatic hypotension and/or urinary incontinence with use of urinary catheters). 70 out of 160 MSA patients (43.8%) developed either severe orthostatic hypotension or urinary incontinence with use of urinary catheters during the course of their illness and 38.1% of MSA patients (n = 61/160) had both features. Although 69.2% of patients with LBD (n = 18/26) had either of these two features, only 7.7% of patients with LBD (n = 2/26) developed both features. None of the PSP patients (n = 0/13) had both features.

References

Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson's disease. Brain Pathol 2010; 20: 633-9.

Adams RD, van Bogaert L, van der Eecken H. Dégénérescences nigro-striées et cérébello-nigro-striées. (Unicité clinique et variabilité pathologique des dégénérescences présénile à forme de rigidité extrapyramidale. Psychiat Neurol Basel 1961; 142: 219-59.

Alafuzoff I, Arzberger T, Al-Sarraj S, Bodi I, Bogdanovic N, Braak H, et al. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol 2008; 18: 484-96.

Anderson T, Luxon L, Quinn N, Daniel S, David Marsden C, Bronstein A. Oculomotor function in multiple system atrophy: clinical and laboratory features in 30 patients. Mov Disord 2008; 23: 977-84.

Aoki N, Boyer PJ, Lund C, Lin WL, Koga S, Ross OA, et al. Atypical multiple system atrophy is a new subtype of frontotemporal lobar degeneration: frontotemporal lobar degeneration associated with α-synuclein. Acta Neuropathol 2015; 130: 93-105.

Brown RG, Lacomblez L, Landwehrmeyer BG, Bak T, Uttner I, Dubois B, et al. Cognitive impairment in patients with multiple system atrophy and progressive supranuclear palsy. Brain 2010; 133: 2382-93.

Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. J Neurosci 2010; 30: 7281-9.

Cykowski MD, Coon EA, Powell SZ, Jenkins SM, Benarroch EE, Low PA, et al. Expanding the spectrum of neuronal pathology in multiple system atrophy. Brain

2015; 138: 2293-309.

Déjerine J, Thomas AA. L’atrophie olivo-ponto-cérébelleuse. Nouv Iconogr Salpêtr 1900; 13: 330-70.

Gilman S, Low PA, Quinn N, Albanese A, Ben-Shlomo Y, Fowler CJ, et al. Consensus statement on the diagnosis of multiple system atrophy. J Neurol Sci 1999; 163: 94-8.

Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008; 71: 670-6.

Goetz CG, Stebbins GT. Risk factors for nursing home placement in advanced Parkinson's disease. Neurology 1993; 43: 2227-9.

Graham JG, Oppenheimer DR. Orthostatic hypotension and nicotine sensitivity in a case of multiple system atrophy. J Neurol Neurosurg Psychiatry 1969; 32: 28-34.

Green J, McDonald WM, Vitek JL, Evatt M, Freeman A, Haber M, et al. Cognitive impairments in advanced PD without dementia. Neurology 2002; 59: 1320-4.

Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967; 17: 427-42.

Höglinger GU, Respondek G, Stamelou M, Kurz C, Josephs KA, Lang AE, et al. Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. Mov Disord 2017; 32: 853-64.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992; 55: 181-4.

Hughes AJ, Daniel SE, Blankson S, Lees AJ. A clinicopathologic study of 100 cases of Parkinson's disease. Arch Neurol 1993; 50: 140-8.

Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 2012; 8: 1-13.

Kaufmann H, Nahm K, Purohit D, Wolfe D. Autonomic failure as the initial presentation of Parkinson disease and dementia with Lewy bodies. Neurology 2004; 63: 1093-5.

Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. Brain 2010;133: 1755-62.

Kimber J, Mathias CJ, Lees AJ, Bleasdale-Barr K, Chang HS, Churchyard A, et al. Physiological, pharmacological and neurohormonal assessment of autonomic function in progressive supranuclear palsy. Brain 2000; 123: 1422-30.

Klos KJ, Ahlskog JE, Josephs KA, Apaydin H, Parisi JE, Boeve BF, et al. Alpha-synuclein pathology in the spinal cords of neurologically asymptomatic aged individuals. Neurology 2006; 66: 1100-2.

Koga S, Aoki N, Uitti RJ, van Gerpen JA, Cheshire WP, Josephs KA, et al. When DLB, PD, and PSP masquerade as MSA: an autopsy study of 134 patients. Neurology 2015; 85: 404-12.

Koga S, Parks A, Uitti RJ, van Gerpen JA, Cheshire WP, Wszolek ZK, et al. Profile of cognitive impairment and underlying pathology in multiple system atrophy. Mov Disord 2017; 32: 405-13.

Koga S, Dickson DW. Recent advances in neuropathology, biomarkers and therapeutic approach of multiple system atrophy. J Neurol Neurosurg Psychiatry 2018; 89: 175-84.

Köllensperger M, Geser F, Seppi K, Stampfer-Kountchev M, Sawires M, Scherfler C, et al. Red flags for multiple system atrophy. Mov Disord 2008; 23: 1093-9.

Low PA, Reich SG, Jankovic J, Shults CW, Stern MB, Novak P, et al. Natural history of multiple system atrophy in the USA: a prospective cohort study. Lancet Neurol 2015; 14: 710-9.

Magalhães M, Wenning GK, Daniel SE, Quinn NP. Autonomic dysfunction in pathologically confirmed multiple system atrophy and idiopathic Parkinson's disease--a retrospective comparison. Acta Neurol Scand. 1995; 91: 98-102.

McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology 2017; 89: 88-100.

Mori H, Oda M, Komori T, Arai N, Takanashi M, Mizutani T, et al. Lewy bodies in progressive supranuclear palsy. Acta Neuropathol 2002; 104: 273-8.

Fanciulli A, Wenning GK. Multiple-system atrophy. N Engl J Med 2015; 372: 249-63.

Okuma Y, Fujishima K, Miwa H, Mori H, Mizuno Y. Myoclonic tremulous movements in multiple system atrophy are a form of cortical myoclonus. Mov Disord 2005; 20: 451-6.

Oliveira MCB, Ling H, Lees AJ, Holton JL, De Pablo-Fernandez E, Warner TT. Association of autonomic symptoms with disease progression and survival in progressive supranuclear palsy. J Neurol Neurosurg Psychiatry. 2019; 90: 555-61.

Osaki Y, Ben-Shlomo Y, Lees AJ, Wenning GK, Quinn NP. A validation exercise on the new consensus criteria for multiple system atrophy. Mov Disord 2009; 24: 2272-6.

O'Sullivan SS, Massey LA, Williams DR, Silveira-Moriyama L, Kempster PA, Holton JL, et al. Clinical outcomes of progressive supranuclear palsy and multiple system atrophy. Brain 2008; 131: 1362-72.

Ozawa T, Paviour D, Quinn NP, Josephs KA, Sangha H, Kilford L, et al. The spectrum of

pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations. Brain 2004; 127: 2657-71.

Quinn N. Multiple system atrophy--the nature of the beast. J Neurol Neurosurg Psychiatry 1989; Suppl: 78-89.

Riku Y, Watanabe H, Mimuro M, Iwasaki Y, Ito M, Katsuno M, et al. Non-motor multiple system atrophy associated with sudden death: pathological observations of autonomic nuclei. J Neurol 2017; 264: 2249-57.

Robinson JL, Lee EB, Xie SX,Rennert L, Suh E, Bredenberg C, et al. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. Brain 2018; 141: 2181-93.

Shy GM, Drager GA. A neurological syndrome associated with orthostatic hypotension: a clinical-pathological study. Arch Neurol 1960; 2: 511-27.

Uchikado H, DelleDonne A, Ahmed Z, Dickson DW. Lewy bodies in progressive supranuclear palsy represent an independent disease process. J Neuropathol Exp Neurol 2006; 65: 387-95.

Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). J Neurol Sci 1989; 94: 79-100.

Papp MI, Lantos PL. Accumulation of tubular structures in oligodendroglial and neuronal cells as the basic alteration in multiple system atrophy. J Neurol Sci 1992; 107: 172-82.

Petrovic IN, Ling H, Asi Y, Ahmed Z, Kukkle PL, Hazrati LN, et al. Multiple system atrophy-parkinsonism with slow progression and prolonged survival: a diagnostic catch. Mov Disord 2012; 27: 1186-90.

Wakabayashi K, Yoshimoto M, Tsuji S, Takahashi H. α-Synuclein immunoreactivity in

glial cytoplasmic inclusions in multiple system atrophy. Neurosci Lett 1998; 249: 180-82.

Williams DR, Lees AJ. Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges. Lancet Neurol 2009; 8: 270-9.

Williams DR, Lees AJ. What features improve the accuracy of the clinical diagnosis of progressive supranuclear palsy-parkinsonism (PSP-P)? Mov Disord 2010; 25: 357-62.

Wenning GK, Tison F, Ben Shlomo Y, Daniel SE, Quinn NP. Multiple system atrophy: a review of 203 pathologically proven cases. Mov Disord 1997; 12: 133-47.

Wenning GK, Scherfler C, Granata R, Bösch S, Verny M, Chaudhuri KR, et al. Time course of symptomatic orthostatic hypotension and urinary incontinence in patients with postmortem confirmed parkinsonian syndromes: a clinicopathological study. J Neurol Neurosurg Psychiatry 1999; 67: 620-3.

Wenning GK, Geser F, Krismer F, Seppi K, Duerr S, Boesch S, et al. The natural history of multiple system atrophy: a prospective European cohort study. Lancet Neurol 2013; 12: 264-74.