Review article

Laboratory supported MSA beyond autonomic function testing and imaging: a systematic review by the MoDiMSA study group

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Abstract

Background: Neuroimaging findings suggestive of multiple system atrophy (MSA) have been used to support the diagnosis of possible MSA. Autonomic function tests (AFT), except for blood pressure change upon standing, although not recognized in the second consensus criteria, are also useful to diagnose widespread and progressive autonomic failure typical of MSA. Additional diagnostic tools are of interest to improve suboptimal accuracy of MSA diagnosis.

Objectives: To assess the utility of diagnostic tools beyond brain imaging and AFT in enhancing a laboratory supported diagnosis of MSA in the upcoming revision of the consensus criteria.

Methods: The Movement Disorders Society MSA (MoDiMSA) study group performed a systematic review of original papers on biomarkers, sleep studies, genetic, neuroendocrine, neurophysiological, neuropsychological and other tests including olfactory testing and acute levodopa challenge test published before August 2019.

Results: Evaluation of history of levodopa responsiveness and olfaction is useful in patients in whom MSA-parkinsonian type is suspected. Neuropsychological testing is recommended to exclude dementia. Applicability of sphincter EMG is limited to selected cases. When MSA-cerebellar type is suspected, we recommend a screening for the common causes of adult onset progressive ataxia including spinocerebellar ataxias in selected patients. Diagnosing sleep abnormalities is useful in both motor MSA subtypes. However, utility of none of these tools is validated in large longitudinal cohorts of postmortem confirmed MSA cases.

Conclusions: Despite limited evidence, an extension of the laboratory work-up of patients with MSA beyond imaging and AFT should be considered to optimize the diagnostic accuracy during lifetime.

Introduction

Multiple system atrophy (MSA) is an adult-onset neurodegenerative disorder manifesting with autonomic failure, parkinsonism and cerebellar ataxia in any combination. Neuropathologically, MSA is a synucleinopathy characterized by abnormal aggregation of alpha-synuclein in glial cytoplasmic inclusions and neurodegenerative changes in striatonigral or olivopontocerebellar structures. Clinical diagnosis of MSA is made according to the consensus criteria built up as a combination of clinical features, and imaging findings, that reflect changes in putamen and infratentorial brain structures such as pons, middle cerebellar peduncle (MCP) and cerebellum.[1] In the current diagnostic criteria for MSA, brain MRI and $^{[18F]}$FDG-PET findings contribute to the diagnosis of possible MSA, whereas the diagnosis of probable MSA is exclusively based on clinical features.[1] Two recent clinicopathological studies have shown that the accuracy of MSA diagnosis during lifetime against neuropathologically established diagnosis ranges between 62% and 79%.[2,3] The previous systematic review by the the International Parkinson and Movement Disorder Society (MDS)-endorsed MSA (MoDiMSA) study group focused the utility of imaging and autonomic function tests (AFT) for the early diagnosis of MSA.[4] Recent data suggest that inclusion of diffusion-weighted MRI sequences and automated volume segmentation to the conventional MRI protocols may allow for an earlier and more accurate diagnosis.[4] However, diagnosis of MSA based on imaging remains challenging due to overlap with Lewy body disorders, such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), progressive supranuclear palsy (PSP), sporadic adult onset ataxia (SAOA) and, less commonly, genetic disorders mimicking MSA. Cardiovascular autonomic tests (excluding blood pressure change upon standing), urodynamic tests and $^{[123I]}$MIBG-scintigraphy are not recognized in the current consensus criteria,[1] although laboratory indices of early, progressive and severe autonomic failure can be useful to improve diagnostic accuracy in individual cases.[4,5] In some MSA patients, autonomic failure may be mild or moderate or may appear later in the disease course, resembling that of Lewy body disorders.[6] Given these limitations, inclusion of diagnostic tools

beyond brain and cardiac imaging and AFT into the revised consensus criteria needs to be considered to enhance a laboratory supported diagnosis of MSA.[7] Therefore, the MoDiMSA study group conducted a systematic review of the literature to determine the accuracy, benefits and limitations of additional diagnostic tests in the work-up of patients with MSA.

Methods

The systematic literature review was conducted by applying prespecified search terms (available for each domain in the Supplementary systematic evidence tables S1-S7) in Pubmed (Medline). Original articles published *in extenso* in English between 1989 and August 1st, 2019 were included if the following inclusion criteria were met: at least ten patients with MSA per study defined either by post-mortem verification, or clinically probable, or clinically probable plus possible MSA according to the consensus criteria,[1,8,9] and at least one reference group of MSA-related disorders, including PD, DLB, PSP, and SAOA. Due to the specific nature of biomarkers and genetic testing, we included, as an exception, studies with unclassified MSA (level of diagnostic accuracy not provided in the paper) and healthy controls as the only comparative group.

Data were extracted using prespecified extraction forms including test domain, authors, publication year, number of patients with MSA and their disease duration, reference group(s), level of diagnostic accuracy, methods, sensitivity, specificity, positive and negative predictive values (PPV, NPV), study results and comments. Results are reported in seven systematic domain-specific evidence tables, including the domains biomarkers, genetic testing, neuroendocrine tests, neurophysiological tests, neuropsychological tests, sleep studies, and other tests including olfactory testing and acute levodopa challenge test. Relevant studies that fulfilled the inclusion criteria were critically analyzed by the MoDiMSA study group experts allocated to working groups on seven diagnostic domains. Working groups' statements on the assigned domains were compiled in a present manuscript.

Results

The search strategy identified 6531 publications. Number of studies screened for each domain is available in the Supplementary systematic evidence tables S1-S7. A total of 235 articles met

the inclusion citeria, including 77 studies on biomarkers, 95 on genetic testing, 7 on neuroendocrine tests, 26 on neurophysiological tests, 14 on neuropsychological tests, 3 on sleep studies, and 13 on other tests (see Supplementary systematic evidence tables S1-S7 for data from individual papers).

Additional tests in patients with parkinsonism suggestive of MSA

Evaluation of levodopa responsiveness

Levodopa responsiveness should be reviewed in newly diagnosed patients with parkinsonism and at regular intervals afterwards. Parkinsonism that is poorly responsive to levodopa is considered a hallmark of MSA.[1] However, a transient, usually modest, levodopa response is documented in a considerable proportion of patients in clinicopathological and natural history studies, with occasional patients experiencing dramatic responses.[10–12] Levodopa unresponsiveness has usually been defined as either <30% improvement on the Movement Disorder Society Unified Parkinson's Disease Rating Scale or Unified Multiple System Atrophy Rating Scale motor examination on up to 1000 mg L-dopa with a peripheral decarboxylase inhibitor for one month, if tolerated, or by applying an acute levodopa challenge test. This test showed poorer response with more frequent side effects upon levodopa administration (e.g., nausea) in patients with MSA compared to patients with PD (Table 1).[13,14]

Olfactory testing

Olfactory testing is easy, cost-effective and non-invasive. It aids in the differential diagnosis of MSA as most patients with PD have hyposmia in contrast to patients with MSA and PSP who have relatively preserved olfaction (Table 1).[15] A combination of hyposmia and an abnormal cardiac sympathetic imaging should guide clinicians towards the diagnosis of PD versus MSA.[16] Fluctuations of olfactory performance that may affect the test's diagnostic value, especially at early disease stages, have been found in a small but relevant fraction of PD patients during observation periods of 4-5 years.[17] Common pitfalls of the smell test include the presence of allergic rhinitis and smoking habits.

Sleep studies

Neuropathological studies have documented that 98% of patients with video-polysomnography (vPSG)-proven REM sleep behavior disorder (RBD) and a neurodegenerative syndrome (parkinsonism or cognitive impairment) have an underlying synucleinopathy.[18] Therefore, documentation of RBD can help to distinguish MSA from non-synucleinopathy neurodegeneration such as PSP but cannot be used to distinguish MSA from Lewy body disorders. RBD can present before MSA onset; a multicenter prospective study found that 8% of patients with idiopathic RBD develop clinically probable MSA.[19]

Other sleep abnormalites are also common in MSA. They include general sleep stage disruption, upper airway dysfunction (apnea and stridor), loss of REM atonia, and periodic leg movements during sleep. MSA patients have more severe loss of REM atonia compared to patients with PD and idiopathic RBD,[20,21] although an overlap between groups limits the diagnostic potential of REM atonia quantification. Evidence for diagnostic utility of other vPSG sleep parameters is limited. Compared to patients with PD and idiopathic RBD, patients with MSA have more periodic leg movements of sleep, more slow-wave sleep, shorter overall sleep duration, and less wake after sleep onset (Table 1).[20] Apnea (i.e., increased apnea/hypopnea index) is commonly observed on vPSG, but not clearly increased compared with other neurodegenerative conditions.[20] Increased snoring is specific for MSA and Lewy body disorders compared to PSP in a clinicopathological series.[3] Symptoms associated with restless leg syndrome are frequent in both patients with MSA and PD.[22]

Inspiratory stridor is commonly observed in MSA and considered a red flag against the diagnosis of PD.[23] Home audio recording is sufficient to make a diagnosis of stridor.[24] Irregular arytenoid cartilages movements were observed on flexible endoscopic evaluation of swallowing in 91% of patients with MSA (of whom, 44% showed clinically overt laryngeal dysfunction with inspiratory stridor), but in no patients with PD in a recent study. [25] In 16% of patients with MSA stridor presents within the first three years from disease onset, indicating low sensitivity in early stages.[26] It has been only rarely documented in other degenerative parkinsonian disorders suggesting high specificity, although controlled studies are lacking.

Pelvic neurophysiology

Evaluation of bladder function in patients with MSA comprises simple (i.e., post-void ultrasonography) and advanced methods such as urodynamic tests and sphincter electromyography (EMG). The post-void bladder ultrasonography is non-invasive, widely available, highly sensitive and specific tool for diagnosing MSA versus PD. However, in early disease stages when symptoms of overactive bladder may be present in both MSA and PD patients, the sensitivity of bladder ultrasonography is suboptimal.[4] Urodynamics is useful for investigation of pathophysiology of urinary retention in patients with MSA.[4]

EMG recordings from the external anal and urethral sphincters are commonly abnormal in MSA.[27,28] In a series of 30 definite MSA cases, 24 had abnormal sphincter EMG, five had borderline results, and only one was normal.[29] Neurogenic changes in MSA occur as a result of involvement of anterior horn cells in the Onuf's nucleus of the sacral spinal cord, and the most consistent abnormalities are prolonged duration of motor unit potentials (MUPs) compared to PD, suggestive of chronic reinnervation (Table 1).[30,31] The value of sphincter EMG in the differential diagnosis of parkinsonism has been debated over the years and a false-negative result can arise if the Onuf's nucleus is yet to be involved. Moreover, automated MUP analysis by the machine tends to exclude long-duration polyphasic potentials with satellite potentials and manual MUP analysis is advisable in cases where MSA is suspected.[32] Similar changes of chronic reinnervation seen in MSA, may be found, though usually to a lesser degree, in long standing PD and other parkinsonian syndromes such as PSP (which also affects Onuf's nucleus), DLB and Spinocerebellar ataxia (SCA) type 3, following cauda equina injury, and following damage to the sphincter muscle such as haemorrhoid surgery and obstetric pelvic floor tears. The prevalence of neurogenic changes increases with duration of disease and worsening neurological disability.[33] A highly abnormal EMG in the absence of other obvious causes in a patient with suspected MSA in the first five years is significant. In contrast, an entirely normal result after five years makes the diagnosis of MSA very unlikely.[29] Lower elicitation rates and prolonged latencies of the bulbocavernosus reflex were observed in patients with MSA compared to patients with PD with early urogenital symptoms (Table1).[34]

Among other neurophysiological tests, auditory startle reflex was occasionally used for identifying PSP (absent or reduced due to pathology in the reticular formation) from MSA (normal response) in small unblinded studies.[35]

Neuropsychological tests

Despite prevalence rates of cognitive impairment of up to 32% in clinical and autopsy confirmed MSA series,[36,37] neuropsychological testing is valuable in the differential diagnosis of MSA and other dementia disorders such as DLB, PD dementia (PDD) and PSP.[38,39] Disproportionate deficits in attention, executive functions and visual processing relative to memory and naming are typical for DLB.[39] Dementia in PD, characterized by frontal-executive dysfunction, initially is mild but can evolve after a mean of ten years from the onset of motor symptoms.[40] In comparative studies patients with DLB and PDD performed worse than patients with MSA across all cognitive domains.[41] In patients with PSP, global cognitive performance is poor compared with MSA patients four years after symptom onset, with more profound executive dysfunction and more rapid progression.[37] One study reported that the Dementia Rating Scale might separate autopsy confirmed MSA from PSP patients with a moderate sensitivity and specificity.[42] The Frontal Assessment Battery (FAB)[43] and Montreal Cognitive Assessment[44] also showed a good discriminative power that was even better for the verbal fluency and Luria series subitems of the FAB (Table 1).[43] More severe deterioration also occurs in other cognitive domains in patients with PSP than in those with MSA.[42,45,46]

A tool specific for the cognitive screening of patients with MSA has not yet been developed. Level-1 examination of the diagnostic procedures for PDD (cognitive deficits severe enough to impact daily living, MMSE<26 and impairment in at least two of the following tests: months backward or serial 7 subtraction, lexical fluency or clock drawing, MMSE pentagons, 3-word recall)[40] showed an excellent specificity of 96.9% and a negative predictive value of 94.1% for detecting dementia in MSA, while a sensitivity of 84.6% was achieved by applying a cut-off MMSE score of 27 instead of 26.[47] Neuropsychological testing is of limited value in the differentiation of patients presenting with ataxia.

Biomarkers

Alpha-synuclein

Decreased α-synuclein levels have been reported in cerebrospinal fluid (CSF) of patients with MSA, but most studies have failed to discriminate between patients with MSA and PD.[48–53] Most recently and outside of the time window of this review, a Real-Time Quaking-Induced Conversion assay was reported to accurately detect α-synuclein seeding activity across Lewy body synucleinopathies but not in MSA.[54] Further research has shown that α-synuclein aggregates associated with PD and MSA corresponded to different conformational strains of α-synuclein[55] and that an α-synuclein-protein misfolding cyclic amplification (PMCA) assay can discriminate between these disorders with an overall sensitivity of 95.4%.[56] In addition, α-synuclein oligomers detected by PMCA analysis together with CSF neurofilament light chain (NfL) were able to discriminate patients with early MSA from those with Lewy body synucleinopathies.[57] Ultrasensitive single molecule array ELISA is another quantification method with a potential in detecting plasma and CSF or exosomal α-synuclein. Inconclusive results on plasma α-synuclein levels in MSA have been reported; some of the variability may be ascribed to the influence of blood contamination, age and different detection procedures.[48,58] Abnormal accumulation of α-synuclein can be caused by dysfunction of the ubiquitin proteasome system, particularly the ubiquitin carboxy-terminal hydrolase L1 enzyme. Reduced levels of the latter have been reported in PD compared to other parkinsonian patients including MSA (Table 1).[59]

Markers of axonal and glial damage

Neurofilament light and heavy chain (NfH) concentrations in CSF are increased in patients with atypical parkinsonism including MSA compared to patients with PD (Table 1).[51,60] Higher NfL levels were also found in serum in patients with atypical parkinsonism including MSA compared to patients with PD, showing good discriminative power in the detection (sensitivity: 82%, specificity: 92%) and validation cohorts (sensitivity: 80%, specificity: 92%), as well as in the cohort of patients with disease duration less than three years (sensitivity: 70%, specificity: 80%), and a strong correlation with CSF levels of NfL.[61]

Amyloid markers

Decreased CSF levels of Aβ1-42, a 42-amino acid long peptide that forms toxic β-amyloid aggregates, and a lower ratio of Aβ1-42/ Aβ1-40, may be used to discriminate patients with DLB from patients with MSA (Table 1).[62,63]

Panels of biomarkers

Combining different wet biomarkers is a promising approach to increase diagnostic accuracy. A set of 9 CSF biomarkers (NfL, sAPPα, sAPPβ, Aβ1-42, total tau, phosphorylated tau, α-synuclein, YKL-40, MCP-1), as well as disease duration and severity were shown to differentiate patients with PD from those with atypical parkinsonism with a sensitivity and specificity of 91%. Among them NfL, α-synuclein and sAPPα independently predicted the diagnosis of PD versus atypical parkinsonism. The same panel was able to differentiate between MSA and PSP patients (Table 1).[49] Serum miR-24, miR-34b, and miR-148b were upregulated in MSA compared to PD in one study.[64]

Arginine stimulation test

The arginine stimulation test is based on the ability of this amino acid to induce growth hormone (GH) secretion through the inhibition of somatostatin release, which is possibly mediated by the cholinergic system. In small unblinded studies it was reported that the GH response to arginine is blunted in patients with MSA, and relatively preserved in patients with PD and PSP.[65]

Genetic screening

An increasing number of heredodegenerative syndromes that can occasionally mimic MSA have been described (Table 2). Among these, a combination of parkinsonism and ataxia may be observed in SCA2, SCA3, SCA6 and SCA17. A complex phenotype with L-dopa unresponsive parkinsonism and central hypoventilation requires attention towards *DCTN1* mutation. In patients of European ancestry, screening for the *C9orf72* hexanucleotide repeat expansion should be considered, especially in cases with a family history of amyotrophic lateral sclerosis or frontotemporal dementia.

Additional tests in patients with ataxia suggestive of MSA

Sleep studies

Adult onset progressive ataxia and autonomic failure, that initially presents with urogenital failure followed by neurogenic orthostatic hypotension (OH), with cerebellar, brainstem and MCP atophy on brain imaging is highly suggestive of MSA-cerebellar type.[66] The presence of RBD in the ataxic patient may point towards the diagnosis of MSA-C versus SAOA (Table 1). In a recent prospective study, probable RBD was present in 83% of MSA-C patients and 11% of SAOA patients.[67] Sleep abnormalities can be also seen in patients with genetic ataxias.[68,69]

Exclusion of common causes of adult onset progressive ataxia

A progressive course of ataxia starting in midlife requires screening for the common causes of cerebellar degeneration including toxic (i.e., alhocol, phenytoin, lithium, barbiturates), metabolic (i.e. vitamine B12, or B1 deficiency syndromes), paraneoplastic and non-cancer related immune mediated disorders (i.e. ataxia associated with antigliadin antibodies, or with anti-glutamic acid decarboxylase antibodies), infections (i.e. cerebellitis), parainfectious syndromes, brain mass lesions and multiple sclerosis.

Genetic screening

Typically, MSA occurs sporadically in the community. Several pathologically confirmed MSA cases occurring in the same family have been reported.[70,71] The diagnostic value of genetic testing in MSA is evaluated in the setting of a suspected monogenic inheritance. Homozygous or compound heterozygous loss-of-function mutations in *COQ2* gene, involved in the coenzyme Q_{10} (COQ_{10}) biosynthesis, are the only monogenic mutations that have been suggested to cause MSA in two Japanese families.[71] Furthermore, the common *COQ2* polymorphism V393A identified in the East Asian populations has been suggested as possible risk variant.[72] This variant is extremely rare in the Caucasian population, which could explain the lack of disease associations in North American or European MSA cohorts.[73,74] Several additional heterozygous variants of unknown significance have been reported in *COQ2*, but their role in disease pathogenesis is unclear and requires further investigation. In addition, decreased concentrations of COQ2 in serum, CSF and cerebellum of MSA patients suggest that COQ_{10} deficiency may contribute to the pathogenesis of MSA (Table 1).[75]

In patients presenting with ataxia, either the presence of a family history or non-supportive features for MSA should guide the physician towards neurogenetic mimicry. These 'red flags', however, may not be present in a given case. Genetic screening, as a second tier after exclusion of the other common causes of midlife onset progressive ataxia, should be considered in selected cases to refine the clinical diagnosis by excluding of the most common mimicries due to the pathogenic mutations in *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *PPP2R2B*, *FMR1,* and *TBP* genes. Repeat expansion mutations in the *RFC1* gene is an underrecognized cause and has been observed in 22% of patients with late-onset cerebellar ataxia.[76] Fragile X–associated tremor/ataxia syndrome resulting from a premutation in *FMR1* gene more frequently poses a differential diagnosis challenge to MSA than the other syndromes due to overlapping clinical and MRI features including hyperintensities in the MCP. In Japanese patients, screening for *DRPLA* needs consideration. Notably, in a large postmortem series, none of the patients with a clinical diagnosis of MSA in life had a final diagnosis of neurogenetic mimic syndrome.[3] Other rare neurogenetic mimic syndromes have been described in the literature, but more detailed discussion goes beyond the scope of this article.

Biomarkers and arginine stimulation test

Patients with MSA-C have increased NfL and NfH levels in CSF compared to patients with SAOA (Table 1).[77]

Arginine GH stmulation test failed to show efficacy in diferentiating between patients with MSA-cerebellar type from patients with SAOA, genetic ataxia and healthy controls (Table 1).[78]

Conclusion and test limitations

Data assessed in this systematic review suggest that several diagnostic tools beyond imaging and AFT may support the diagnosis of MSA in individual cases. The diagnostic discrimination of each tool depends on the clinical context (i.e., predominant clinical presentation and differential diagnosis) and changes over the disease course. The paucity of studies in patients with MSA presenting with isolated autonomic failure (i.e., distinguishing premotor MSA from pure autonomic failure due to Lewy body disease) prevented us from analyzing the evidence in this specific population. A major limitation of the available evidence is an absence of

postmortem diagnostic confirmation in the majority of studies. Because most studies were cross-sectional, including patients with advanced disease stages, the evaluation of the test performance in the first 2-3 years from onset (when the sensitivity for a diagnosis of MSA is most required) is poor.

In patients presenting with parkinsonism, the history of the levodopa response is required, as a poor response to levodopa is characteristic of MSA compared to PD. Studies addressing the performance of an acute levodopa challenge test were difficult to interpret, as they analyzed different MSA populations, and used different levodopa doses, assessment methodologies and outcome measures. There is no information on the value of an acute levodopa challenge in *de novo* MSA patients. Given the methodological diversity and the proportion of MSA patients in early disease stages with levodopa-responsive parkinsonism, we conclude that the acute levodopa challenge test cannot assist in the earlier diagnosis of MSA. Consequently, a negative levodopa challenge – when available – should not deter clinicians from initiating chronic levodopa maintenance therapy, until a daily dose of 1000 mg has been tried for at least a month if needed and tolerated. The moderate discriminative power of olfactory testing in distinguishing MSA (where the test is normal) from PD (where olfaction is typically impaired) suggests that it might be useful to support a diagnosis of MSA, despite a lack of blinded data. There are no studies on the efficiency of combined olfactory testing and cardiac sympathetic imaging in differentiating MSA from PD+OH. Otherwise unexplained neurogenic findings in sphincter EMG within a few years from disease onset are suggestive of MSA. However, due to overlapping denervation patterns between MSA and PD such changes may not support the diagnosis in individual patients. Given some denervation in healthy subjects, the test should be interpreted with caution. Limitations of the sphincter EMG include discomfort for the patient, difficulties in interpreting the results, effects of age, sex, multiple childbirths, and comorbidities such as prostate hypertrophy, bladder neck stenosis, or stress incontinence.

Careful neuropsychological screening is useful to exclude dementia, which is, based on current evidence, exceptionally rare in MSA but is an essential feature of DLB and PDD. Assessment of global cognitive functions employing the Dementia Rating Scale, or executive functions by applying the FAB may aid differentiating patients with MSA from patients with PSP. However,

differentiation made on the basis of cognitive state is not likely to be helpful in early stages. There is a need to define a specific cognitive battery with tests whose performance would not be affected by motor disability.[79]

VPSG-documented RBD and severe loss of REM atonia are highly indicative of a neurodegenerative synucleinopathy such as MSA; hence, their absence makes a diagnosis of MSA unlikely. Documentation of inspiratory stridor by home audio recording or vPSG is very specific for MSA.[24] Based on a small number of relevant studies we conclude that vPSG is useful to distinguish patients with MSA from patients with tauopathies and sporadic, symptomatic and genetic ataxias (although RBD has been documented in selected disorders such as SCA3). VPSG cannot assist in the differential diagnosis of MSA vs. other synucleinopathies.

In patients presenting with progressive adult onset ataxia, immune mediated (including paraneoplastic and non-cancer related disorders), metabolic, toxic, and infectious/postinfectious causes should be excluded. As a second tier, genetic screening for the most common SCAs is recommended, particularly in cases with positive family history or non-supportive features for MSA. Other rare MSA neurogenetic mimicries have been described in the literature (Table 2) but broad genetic testing beyond the common SCAs is currently not recommended.

Although there are several promising biomarker candidates such as α-synuclein or NfL in CSF and plasma, none of them is sufficiently robust to support a diagnosis of MSA. By applying panels with multiple biomarkers, diagnostic accuracy could be improved. The high variability of findings on fluid biomarkers across the literature highlights the need to standardize analytical methods and harmonize standard operating procedures. The validation of current biomarkers in large prospective studies is needed before any wet biomarker could be used for MSA. The arginine growth hormone stimulation test provided confilicting results in different MSA cohorts. The role of this and other neuroendocrine tests remains to be defined in future, larger studies.

As all systematic reviews, this study has several limitations. First, we may have missed original studies due to potential publication bias. Second, we did not report uncertainty of accuracy data, for example, 95% confidence intervals sensitivity and specificity. Third, PPV and NPV must

be judged in the light of prevalence, which was not available for most settings. Fourth, data on the same patients could be published in more than one study; hence, the cumulative number of patients in studies from which the range of diagnostic accuracy measures was derived (Table 1) may not be correct. Fifth, diagnostic test accuracy characteristics alone are not sufficient to inform clinical decision making. Further aspects including the benefits and harms of patients with false negative and false positive results as well as cost effectiveness must be included in the decision-making process, which may require decision-analytic modeling approaches.[80]

In summary, current best evidence suggests that in patients with parkinsonism suggestive of MSA, evaluation of history of levodopa responsiveness and olfactory function is useful. Neuropsychological testing should be performed to exclude dementia. When MSA-cerebellar type is suspected, a screening for the common causes of adult onset progressive ataxia is useful in selected patients. Genetic screening beyond the most common SCAs is not currently recommended. Diagnosing sleep abnormalities is useful in both motor MSA subtypes. The results of neurophysiological tests should be interpreted with caution, and the role of this testing is limited. Based on the current evidence, we conclude that the laboratory work-up should be extended beyond brain and cardiac imaging and autonomic function tests in selected patients with MSA to improve diagnostic accuracy during lifetime. Cohort studies enrolling patients with MSA within first 2 years from symptom onset with blinded evaluations of the test performance and postmortem diagnostic confirmation are required to generate sufficient evidence on early disease stages.

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