EDITORIAL

Autonomic function testing in multiple system atrophy: a prognostic biomarker? and other updates on recent autonomic research

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Autonomic function testing in multiple system atrophy: a prognostic biomarker?

The diagnosis of multiple system atrophy (MSA) is a difficult one to make with confidence, given overlapping clinical features with Parkinson's disease (PD), dementia with Lewy bodies (LBD), and other non-synuclein degenerative disorders, such as progressive supra-nuclear palsy (PSP) or spinocerebellar ataxia (SCA). It is also a diagnosis that no clinician wants to make with confidence, given the poor prognosis and lack of disease-modifying therapies. For these reasons, more reliable biomarkers are urgently needed.

In their article entitled "Diagnosing Premotor Multiple System Atrophy: Natural History and Autonomic Testing in an Autopsy Confirmed Cohort,"[1] Vichayanrat et al. aimed to better characterize early symptoms of MSA to better inform understanding of disease progression and to evaluate the influence of non-motor symptoms on prognosis. They retrospectively reviewed 47 autopsy-confirmed MSA cases (21 MSA-P, 24 MSA-C) from the Queen Square Brain Bank in London between 1992 and 2012. All patients had undergone standardized autonomic cardiovascular reflex testing (heart rate variability with deep breathing, Valsalva maneuver (VM), 10-min head-up tilt (HUT) at 60°). Supine and upright norepinephrine (NE) levels were measured in 20/47 patients. Orthostatic hypotension (OH) was defined as a drop of > 30 mmHg in systolic blood pressure or > 15 mmHg in diastolic blood pressure on HUT (MSA second consensus criteria).

Patients were divided into non-motor onset and motor onset, based on initial presenting symptoms. Examples of

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non-motor symptoms include those of autonomic dysfunction (orthostatic intolerance, bladder dysfunction, erectile dysfunction, gastrointestinal dysfunction) and sleep disorders (symptoms suggestive of REM sleep behavior disorder (RBD) or stridor). Nearly one-third of patients in this cohort presented with non-motor symptoms, and nearly all presented with symptoms of autonomic dysfunction (one presented with symptoms of RBD).

Sixty-eight percent of patients with MSA presented with motor symptoms, while 32% of patients presented with non-motor symptoms. In those with motor presentations, urinary symptoms developed within a median of 3 years and autonomic cardiovascular symptoms within a median of 4 years. All patients eventually developed urinary symptoms. In those with non-motor presentations, erectile and bladder dysfunctions were most common (47% and 40%, respectively—60% of patients were male). Motor symptoms (parkinsonism or cerebellar features) developed within a median of 3 years. One-third of the non-motor patients were initially diagnosed with pure autonomic failure (PAF), a common prodromal diagnosis in MSA. Indeed, OH was present in most patients (100% of patients with non-motor presentation and 75% of patients with motor presentation). Interestingly, symptoms suggesting RBD (unclear if confirmed with polysomnography) were more commonly reported in patients with motor onset (odds ratio 4.3; 95% CI 1.00–18.36, $p = 0.05$).

Patients with isolated sympathetic impairment were younger and had an earlier onset of disease compared to those with generalized cardiovascular autonomic dysfunction. In the 20 patients who had NE measurements during tilt, the average supine value was $345.0 + 145.6$ pg/ml, findings in line with prior studies demonstrating normal plasma norepinephrine levels in MSA, vs. low levels in PAF [2]. The authors also found a minimal rise in plasma norepinephrine with HUT (increase of 34.7 ± 51.0 pg/ml), and this finding

was more common in patients presenting initially with the PAF phenotype. Survival was similar between the motor and non-motor presentation groups, with average disease duration (symptom onset to death) of 8 years. Early cardiovascular autonomic impairment portended a worse prognosis, as was early urinary catheterization and later age of symptom onset.

This study confirms the value of formal autonomic cardiovascular reflex testing in informing the correct diagnosis of MSA. Most patients fit the classic paradigm of sympathetic and parasympathetic failure, however, some patients had isolated parasympathetic failure and 6% had normal autonomic testing on presentation, highlighting the fact that autonomic impairment may not always be present on initial evaluation. Limitations of this study include its retrospective nature, lack of polysomnograms in all patients (most patients likely had RBD and stridor; however, this was not systematically assessed in all patients), and the lack of postganglionic autonomic sudomotor function testing. Other non-motor features, such as olfaction and cognitive function, were also not assessed, as they have been in other longitudinal natural history studies [2]. It would also have been interesting to evaluate resting heart rate and heart rate changes on HUT, as this has been evaluated as a potential biomarker in distinguishing MSA from PAF [2]. Skin biopsy analyses may have also added value. Finally, the cause of death was not assessed, as this may inform the prognostic value of the authors' findings. Nonetheless, the size of the post-mortem cohort is a significant strength, and these findings add to our understanding of MSA and provide additional tools for the autonomic neurologist to more accurately diagnose patients with this condition.

Cranial parasympathetic outflow—time to learn the details

Molecular technologies are improving our understanding of classically defined autonomic pathways. Previously some of these developments have resulted in a paradigm shift in thinking of classically defined autonomic pathways including the possibility that the sacral parasympathetic outflow could potentially primarily be a sympathetic pathway [3], a topic that was debated in a prior *CAR* review [4].

In a recent publication, Veerakumar et al. published their analysis of parasympathetic pathways for cardiovascular and cardiopulmonary function in the Journal *Nature* titled "Molecularly defined circuits for cardiovascular and cardiopulmonary control" [5]. They used multiple molecular biology technologies including retrograde neuronal tracing, single-cell RNA sequencing, opto-genetics, and physiological experiments to tease out different parasympathetic pathways originating in the nucleus ambiguous in mice.

To isolate the cardiac and cardiopulmonary parasympathetic neurons in the nucleus ambiguus, the researchers retrogradely labeled selected neurons by injecting fluorescent cholera toxin into the pericardial space or cricothyroid laryngeal muscle and identified parasympathetic neurons in the nucleus ambiguus. With single-cell RNA sequencing of these neurons, they were able to identify 3 different clusters, two clusters of primarily cardiac neurons and one cluster of cardiopulmonary neurons. All the neurons expressed high levels of cholinergic markers and cranial motor neuron transcription factor, confirming the primary parasympathetic role. Additional immunostaining with calbindin and butyrylcholinesterase revealed that these 2 subtypes (cardiovascular and cardiopulmonary) are different with very minimal crossover.

To understand the differential innervation of cardiovascular neurons from cardiopulmonary neurons, the authors injected rostral ambiguus nucleus neurons with adeno-associated virus-encoding fluorescent protein eYFP, which labeled only the cardiopulmonary neurons. Three weeks after this injection, eYFP-positive fibers were found innervating cardiac ganglionated plexus (GPs). Fibers from the single side of the brainstem provided all the cholinergic input for the given target without any mixing of sides. Similar experiments were also performed for the cardiovascular neurons. They concluded that both cardiopulmonary and cardiovascular neurons innervate cholinergic cardiac ganglionated plexus (GPs). Left and right cardiopulmonary neurons innervate the same subset of GPs and left and right cardiovascular neurons innervate different GPs.

Further physiological evaluation of these nerves included opto-genetic stimulation and such stimulation of the bilateral cardiovascular neurons led to bradycardia. However, stimulation of the left-sided cardiopulmonary neurons caused bradycardia as well as second-degree AV block, whereas stimulation of the right-sided cardiopulmonary neurons only caused bradycardia without AV block. All effects were reversed with atropine. The final physiological assessment was done through baroreflex activation with phenylephrine infusion or by activation of the dive reflex. As expected, baroreflex function testing only activates the cardiovascular neurons whereas the dive reflex predominantly activates the cardiopulmonary neurons. We encourage the reader to review the article for further details regarding different techniques.

In conclusion, this extensive analysis provides evidence that cardiac parasympathetic function is not a single outflow tract but consists of two different pathways with highly different anatomical, functional properties, and transcriptional differences in the neurons. The identification of separate bundles of cardiovascular and cardiopulmonary neurons might have therapeutic interventions. For example, future therapeutics could have preferential or isolated effects on the

cardiovascular pathway but not the cardiopulmonary neurons. We expect that this type of multimodal investigation of classically understood autonomic pathways will unlock some of the mysteries of the autonomic field and lead to therapeutic interventions for our patients.

Declarations

Conflict of interest None.

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