SUPPLEMENTAL MATERIAL

Spinal Arteriolosclerosis Is Associated with Parkinsonism in Older Adults

SUPPLEMENTAL METHODS

Assessment of Parkinsonism: Trained nurse clinicians administered a 26-item modified version of the motor portion of the United Parkinson's Disease Rating Scale (mUPDRS) as previously described. Four previously established parkinsonian sign scores were derived from the 26 items assessed. Parkinsonian gait was based on 6 items, rigidity was based on 5 items, bradykinesia was based on 8 items, and tremor was based on 7 items. [1-3]

Summarizing the Assessment of Parkinsonism: Global Parkinsonian Score: Each of the 4 parkinsonian sign scores (parkinsonian gait, bradykinesia, rigidity and tremor) were calculated by adding the number of points assigned to each of the individual items which were rated e.g., 8 items for bradykinesia. The total number of points for these 8 items is the raw bradykinesia score. This score was then divided by the maximal possible score which could be given for the domain which in the case of bradykinesia is 40 (8x5=40). This ratio (actual points/maximal points) was then multiplied by 100. Thus, while the number of items varied for each of the 4 parkinsonian signs, each the four signs were scaled from 0 to 100. This procedure was done to calculate a score for each of the four parkinsonian signs. A summary global parkinsonian sign score was constructed by averaging the four individual parkinsonian sign scores. [1-3]

Demographics, Global Cognition and Self-Reported Medical Conditions

Age, sex and years of education were recorded at baseline interview. Nineteen cognitive tests were administered and summarized as a composite measure of global cognition. The minimental status score was used for descriptive purposes and was not included in the global score of cognition. [1] As previously described, the number of 3 self-reported vascular risk factors (i.e. hypertension, diabetes mellitus, and smoking), and the number of 4 self-reported vascular diseases (i.e., myocardial infarction, congestive heart failure, claudication and stroke) were used in these analyses. [1] Cervical radiculopathy was based on self-report by the participant of neck pain as well as well as pain or sensory disturbances which concurrently affected the arm, forearm or hand ipsilateral to the cervical pain.

Post-Mortem Indices

At the time autopsy spinal cord and brains were removed, weighed, and regions that were not designated for freezing were immersion fixed in 4% paraformaldehyde for a minimum of 72 hours.

Spinal Arteriolosclerosis Composite Measure

Spinal arteriolosclerosis measures are summarized in Table e1. The severity of anterior and posterior SVD differs across the 4 spinal levels (Friedman's test, Anterior: X^2=24.3, dF=3, p<0.001; X^2=71.8, DF=3, p<0.001). We then employed pairwise Wilcoxon signed rank tests with Bonferroni correction to determine which pairs of levels differed. There were no significant differences between the cervical and lumbar levels (p=0.207), cervical and thoracic levels (p=0.375) and lumbar and thoracic levels (p=0.728). However, spinal arteriolosclerosis was less severe at the sacral level than any of the other three cord levels (p<0.001 for each pair). Nonetheless, while sacral spinal arteriolosclerosis was less severe, sacral spinal arteriolosclerosis severity was still

correlated with spinal arteriolosclerosis severity at the other three cord levels (average r=0.40).

These findings supported combining severity assessments from all 4 spinal cord levels into a composite spinal arteriolosclerosis severity measure. The composite measure of spinal arteriolosclerosis was based on the mean of 8 values including the anterior and posterior severity assessments from each of the 4 spinal levels.

Spinal White Matter Pallor Composite Measure

Spinal white matter pallor measures are summarized in Table e4. Friedman's non-parametric test showed that there was a significant differences between the severity of gracilis white matter pallor at the 4 spinal levels DF=3, X^2=176.6, p<0.001.

We then employed pairwise signed rank tests to determine which levels differed. There were significant differences between the cervical-lumbar (Signed Rank p<0.001), cervical-thoracic (Signed Rank p<0.001) and cervical-sacral levels (Signed Rank p<0.001) lumbar-sacral levels (Signed Rank p<0.001) thoracic-sacral levels (Signed Rank p<0.001) but not at the lumbar-thoracic level (Signed Rank p=0.274).

Friedman's non-parametric test showed that there was a significant differences between the severity of LCST white matter pallor at the 4 spinal levels DF=3, X^2=15.3, p=0.002. We again employed pairwise signed rank tests to determine which levels differed. There were significant differences between the cervical-sacral levels (Signed Rank p=0.015) and lumbar-sacral levels (Signed Rank p<0.001) but not at the cervical-lumbar (Signed Rank p=436), cervical-thoracic (Signed Rank p=0.115) thoracic-sacral levels (Signed Rank p=0.126) but not at the lumbar-thoracic level (Signed Rank p=0.052). However, white matter pallor was less severe midline at the sacral level than all three other cord levels (p<0.001 for each pair). Nonetheless, while sacral spinal arteriolosclerosis was less severe, midline sacral white matter pallor was still correlated with white matter pallor severity at the other three cord levels (average r=0.23).

These findings supported combining severity assessments from all 4 spinal cord levels into a composite white matter pallor severity measure. A mean white matter pallor score was calculated by averaging the individual white matter pallor scores for each spinal level (Table e4 below). In the cervical spinal level this included the severity of the Cuneatus, Gracilis and LCST. In the other 3 spinal levels this included the Gracilis and LCST. The composite spinal white matter pallor measure was constructed by averaging the mean white matter pallor scores of all 4 spinal levels together.

Other Cerebrovascular Disease Pathologies

Macroscopic Cerebral Infarcts: We reviewed 1 cm slabs and recorded the age, volume (in mm^3), side, and location of all cerebral infarcts visible to the naked eye as previously reported. There was no minimum size required for macroscopic infarcts. All grossly visualized and suspected macroscopic infarcts were microscopically reviewed for histologic confirmation. Infarct age (acute, subacute and chronic) was estimated by gross histologic features, degree of cavitation and microscopy. [1]

Cerebral Atherosclerosis: This describes the segmental or circumferential subintimal accumulation of lipid, plasma proteins and calcium deposition (plaque) in the walls of large arteries. It was assessed on gross examination of the anterior, middle, and posterior cerebral arteries and their proximal branches at the circle of Willis using a semiquantitative scale of none (zero) to blocked (6) indicating near total or total involvement of all visualized arteries. [1]

Alzheimer's Pathology

Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles in the midfrontal, midtemporal, inferior parietal, and entorhinal cortices, and the hippocampus, as previously described. Briefly, the operator used a graticule to project a grid to count numbers of each pathological marker in a $1mm^2$ area (magnification x100) under the microscope. Counts were performed by a board-certified neuropathologist or trained technician blinded to all clinical data. [1]

Plaques and tangle counts had different ranges and were not normally distributed; therefore, we created standardized scores for each plaque and tangle count in each cortical area as previously described. These scaled scores for each region were then averaged across the five regions (midfrontal, midtemporal, inferior parietal, entorhinal cortices and hippocampas) to develop summary scores for diffuse plaques, neuritic plaques, and neurofibrillary tangles for each subject. We then averaged the summary scores of the three AD markers to yield the global measure of AD pathology for each subject used in these analyses. [1]

PD Pathology

Dissection of diagnostic blocks included a hemisection of midbrain which included substantia nigra. *Nigral neuronal loss* was assessed in the substantia nigra in the mid to rostral midbrain near or at the exit of the 3rd nerve using H&E stain and 6 micron sections using a semi-quantitative scale (0-3). *Lewy body disease pathology* was identified with a monoclonal antibody to phosphorylated alpha-synuclein (1:20,000, Wako Chem Inc., Richmond, VA). [1]

Table I. Semiquantitative assessment of spinal arteriolosclerosis						
Mean Severity of Spinal Cord Arteriolosclerosis (0-6)				Case by Case Severity Anterior vs Posterior N (%)		
Variable	Anterior	Posterior	Difference	Anterior > Posterior	Anterior = Posterior	Anterior < Posterior
Cervical	3.9 (0.92)	4.5 (0.79)	0.7 (0.72)	3 (2%)	69 (43%)	88 (55%)
Thoracic	4.0 (0.91)	4.4 (0.91)	0.4 (0.60)	3 (2%)	93 (64%)	50 (34%)
Lumbar	3.9 (0.97)	4.5 (0.85)	0.6 (0.75)	4 (3%)	77 (49%)	76 (48%)
Sacral	3.6 (0.94)	3.7 (0.94)	0.1 (0.49)	6 (4%)	107 (79%)	22 (16%)

Table II. Associations of spinal cord and brain arteriolosclerosis with other brain neuropathologies (N=165)				
	Spinal Cord		Brain	
Brain Neuropathology	r	p	r	p
Macroinfarcts	0.072	0.358	0.134	0.081
Microinfarcts	0.019	0.812	0.170	**0.026**
Atherosclerosis	0.098	0.212	0.134	0.081
Cerebral amyloid angiopathy	-0.075	0.336	-0.014	0.858
Alzheimer's disease pathology	-0.054	0.491	-0.074	0.334
Lewy body pathology	0.035	0.655	-0.013	0.870
Nigral neuronal loss	0.066	0.395	0.086	0.261

Table III. Spinal cord and brain arteriolosclerosis and risk factors				
Risk Factor	Spinal arteriolosclerosis		Brain arteriolosclerosis	
	R	p	r	P
High BMI	0.157	**0.046**	0.113	0.145
Hypertension	0.054	0.493	0.093	0.227
Diabetes	-0.071	0.366	0.045	0.561
Smoking	-0.158	**0.044**	-0.099	0.197
Claudication	-0.004	0.964	-0.003	0.862
Myocardial infarction	0.067	0.389	-0.022	0.776
Congestive heart failure	-0.027	0.739	0.026	0.744

Table IV. Severity of Spinal White Matter Pallor (0-6)*						
				Case by Case Severity of LCST* vs Gracilis N (%)		
Cord Level	Cuneatus	Gracilis	LCST	LCST > Gracilis	LCST = Gracilis	LCST < Gracilis
Cervical	3.21 (1.04)	4.67(1.02)	2.82 (0.95)	4 (2%)	14 (9%)	142 (89%)
Thoracic	--	4.12 (1.24)	2.69 (0.94)	5 (3%)	22 (15%)	120 (82%)
Lumbar	--	4.36 (1.06)	2.88 (0.84)	3 (2%)	21 (13%)	133 (85%)
Sacral	--	2.79 (0.91)	2.49 (0.72)	10 (7%)	83 (61%)	43 (32%)

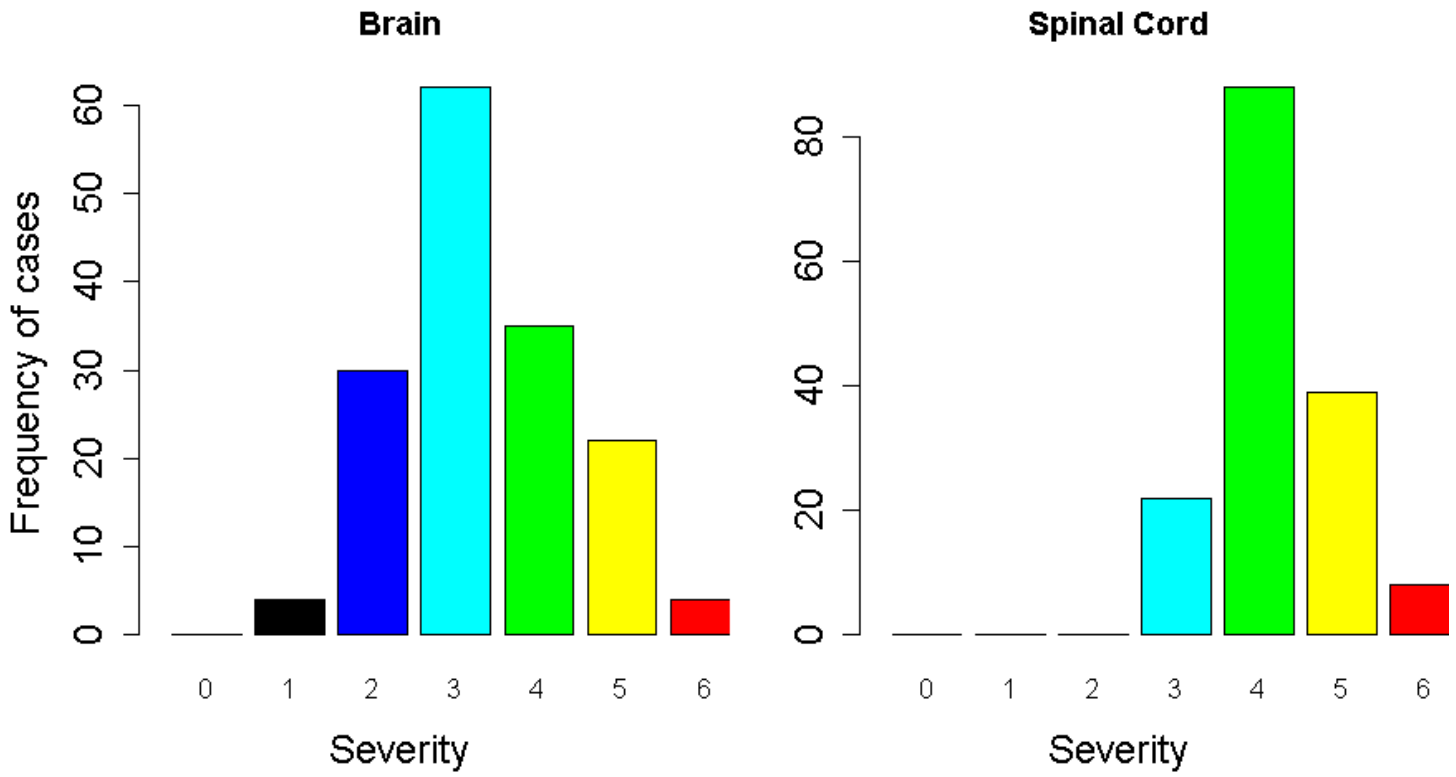
***LCST** – Lateral corticospinal tract.

Table V. Spinal arteriolosclerosis, other brain pathologies and parkinsonism proximate to death excluding cases with a history of PD

Postmortem Indices	Term	Model A			Model B			Model C			Model D			Model E		
		Est.	S.E	Sig.	Est.	S.E	Sig.	Est.	S.E	Sig.	Est.	S.E	Sig	Est.	Est.	Est.
Arteriolosclerosis	Spinal arteriolosclerosis	0.388	0.146	0.009				0.341	0.149	0.023	0.356	0.156	0.016	0.390	0.147	0.009
	Brain arteriolosclerosis				0.204	0.104	0.053	0.151	0.105	0.155						
Brain Cerebrovascular Pathologies	Macroinfarcts										0.108	0.216	0.617			
	Microinfarcts										-0.411	0.215	0.058			
	Cerebral Amyloid (CAA)										-0.092	0.090	0.310			
	Atherosclerosis										0.309	0.179	0.085			
Brain Degenerative Pathologies	Lewy body pathology													0.028	0.274	0.920
	Nigral neuronal loss													0.075	0.158	0.635
	AD pathology													0.38	0.165	0.404
	Adjusted R- squared	3.90%			1.90%			4.55%			6.28%			2.69%		

Each column (A-E) represents a separate regression model with the *Estimate* (**Est.**), *Standard Error* (**S.E**) and *p-Value* (**Sig**.) for each predictor with parkinsonism as the outcome. These models included spinal arteriolosclerosis as the predictor alone (A) and additional models controlling for postmortem indices of other brain pathologies (C-E). All models included terms controlling for age, sex, time from last exam to death (results are not shown). **Adjusted R- squared** shows the percentage of the variance of parkinsonism due to the postmortem indices included in each model after subtracting the percentage variance due to age, sex and the time from the last exam to death.

Figure I. Heterogeneity of arteriolosclerosis in spinal cord and brain.



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