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ORIGINAL COMMUNICATION

Spinal motor neurons and motor function in older adults

Aron S. Buchman[1,2] · Sue E. Leurgans[1,2] · Veronique G. J. M. VanderHorst[3,4] · Sukriti Nag[1,5] · Julie A. Schneider[1,2,5] · David A. Bennett[1,2]

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Abstract

This study examined the relation between lumbar spinal motor neuron (SMN) indices and motor function proximate to death in community-dwelling older adults. Older adults ($N = 145$) participating in the Rush Memory and Aging Project underwent structured clinical testing proximate to death and brain and spinal cord autopsy at time of death. Ten motor performances were summarized by a composite global motor score. Choline acetyltransferase immunostaining was used to identify spinal motor neurons of the L4/5 segment. SMN counts and area and ventral horn area were collected. Linear regression modeling showed that the association of SMN counts and density with global motor scores proximate to death varied with sex. Separate models in men and women showed that this significant interaction was due to the association of higher SMN counts and density with higher global motor scores proximate to death in men but not women. These associations were unchanged when we controlled for indices of brain pathologies or chronic health conditions. In 38 cases with counts of activated microglia available, higher counts of activated microglia were associated with lower SMN counts. Activated spinal microglia and loss of spinal motor neurons may contribute to motor impairments in older men.

Keywords Aging · Motor function · Spinal motor neurons · Spinal microglia

Abbreviations

SMN	Spinal motor neuron
CNS	Central nervous system
PNS	Peripheral nervous system
AD	Alzheimer's disease
TDP-43	TAR DNA-binding protein 43
BMI	Body mass index

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✉ Aron S. Buchman
aron_s_buchman@rush.edu

[1] Rush Alzheimer's Disease Center, Rush University Medical Center, Jelke Building, Suite #1000; 1750 West Harrison Street, Chicago, IL 60612, USA

[2] Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA

[3] Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA

[4] Harvard Medical School, Boston, MA, USA

[5] Department of Pathology (Neuropathology), Rush University Medical Center, Chicago, IL, USA

Introduction

Loss of motor function is common in older adults and associated with diverse adverse health outcomes [1, 37]. Understanding its pathologic basis is crucial for efforts to prevent its development. Healthy motor function depends on diverse systems such as musculoskeletal structures, cardiopulmonary systems, metabolism and neural systems [2, 18, 22, 23, 25, 37, 38, 46]. Neural control systems underlying movement originate in the brain and extend via brainstem to influence spinal motor neurons (SMN) in the spinal cord which activate muscle contractions, the final effector of all movement.

While there is increasing recognition of the importance of CNS contributions to motor impairment in older adults, most prior studies have focused on the role of accumulating brain pathologies or on sarcopenia, the loss of muscle structure and function due to late-life motor impairment. Despite the crucial role of spinal motor neurons for all movement, there are limited data examining their association with motor performance assessments obtained proximate to death in older adults. In contrast to brain imaging or muscle histopathology, spinal cord tissues cannot be assessed in living older adults. Obtaining spinal motor neuron counts is laborious

and not routinely done at autopsy. Postmortem human studies in adults without neurologic disease suggest that there is a loss of spinal motor neurons with increasing age [19]. However, these studies have generally been limited to the collection and examination of spinal motor neuron indices without clinical motor testing results available proximate to death.

To fill this gap, we used clinical and postmortem data obtained prospectively, from older adults participating in the Rush Memory and Aging Project, a community-based cohort study of chronic conditions of aging in which participants agree to annual clinical assessments and autopsy at time of death [4]. First, we examined the distribution of lumbar spinal motor neuron (SMN) indices in older adults. Then, we examined the relationship of lumbar SMN indices with indices of other age-related brain pathologies which were available. Recent studies suggest a role for microglia in the loss of spinal motor neurons in motor neuron disease [33]. Therefore, in a subset of cases, we obtained activated spinal microglia counts in the ventral horns where from which we obtained spinal motor neuron indices. Then, we examined the relationship of SMN indices with the level of motor function proximate to death.

Methods

Participants

Participants were individuals from the Memory and Aging Project, an ongoing community-based cohort study of aging [4]. Although the Memory and Aging Project began in 1997, the substudy to collect spinal motor neuron data was not added till 2005. There were 145 cases collected prospectively with clinical data available proximate to death which was analyzed in this study. The study was approved by the Institutional Review Board of Rush University Medical Center. Written informed consent was obtained from all study participants.

Assessment of motor function

Ten motor performances are assessed as part of a uniform structured clinical evaluation [4]. (1) Grip and (2) pinch strength were measured bilaterally using the Jamar® hydraulic hand and pinch dynamometers (Lafayette Instruments, Lafayette) to assess manual strength. Upper extremity dexterity was based on (3) the number of pegs that could be placed into holes in a pegboard (Purdue Pegboard) in 30 s. Two trials were recorded for each hand. The four trials were averaged to provide a Purdue Pegboard score. In addition, (4) participants tapped an electronic tapper (Western Psychological Services, Los Angeles, CA, USA) with their index finger as quickly as possible for 10 s. Two trials were performed for each hand. The four trials were averaged together to yield a tapping score. To evaluate gait, we asked people to walk eight feet and turn 360° and measured the (5, 6) time and (7, 8) number of steps taken on each task. (9) To assess balance, we asked people to stand on each leg for 10 s. (10) Persons were asked to stand on their toes for 10 s.

These measures were scaled and averaged to obtain a summary global motor score as previously described [5, 45]. This composite global motor score has been previously reported to be associated with risk of mortality, incident disability and dementia [5]. In addition, we have shown that diverse indices of brain pathologies are associated with declining global motor scores proximate to death [8, 13, 15].

Other covariates

Age was computed from self-reported date of birth. Sex and years of education were recorded at the study entry. A summary measure of global cognition was based on 17 cognitive tests [4]. Body mass index was based on measured weight and height [4]. The numbers of four self-reported vascular diseases and three self-reported vascular risk factors were used in these analyses (Online Resource 1 Methods) [4].

Postmortem assessment of brain and lumbar spinal cord specimens

On death, the brain and spinal cord were dissected. One cerebral hemisphere, the brain stem and spinal cord were placed in 4% paraformaldehyde in 0.1 M phosphate buffer for a minimum of 48 h prior to further processing and histopathologic evaluation [4].

Assessment of brain indices

A uniform gross and microscopic exam with quantification of postmortem indices in the brain included summary measure of AD pathology, other neurodegenerative pathologies (TDP-43, Lewy Bodies, hippocampal sclerosis, nigral neuronal loss) and cerebrovascular disease pathologies (chronic macroinfarcts and microinfarcts, cerebral amyloid angiopathy, atherosclerosis and arteriolosclerosis). (Online Resource 1 Methods) [20].

Assessment of lumbar spinal motor neurons

ROI and staining SMN

The entire cord was removed and the lumbosacral enlargement (about L1–S2), the widest area of lumbar cord with a corresponding increase in diameter of the ventral roots was cut at the approximate midpoint (approximately L4).

On either side of the cut, 5-mm-thick cross-sections of cord were frozen and stored for further studies. A 5 mm section above and below were then, fixed and processed using routine techniques and paraffin-embedded to obtain approximate levels of L3–4 and L4–L5. Serial sections (40 µm) of the L4/5 segment were cut to ensure that even with tissue shrinkage there would be adequate tissue to employ guard volumes (4–5 µm) and to ensure adequate batch penetration. Four slides/block were examined for each case which were taken from the middle of the block separated by a distance of 400 µm. Immunohistochemistry was performed using a Bond automated immunostainer (Leica Biosystems, New Buffalo, IL, USA). Sections were placed in Bond heat-induced epitope retrieval solution 1 (Leica Biosystems) for 30 min followed by immunostaining with a monoclonal antibody to choline acetyltransferase (ChAT, 1:50 dilution, Novocastra Laboratories, New-castle-upon-Tyne, UK) which identifies spinal motor neurons. The Bond Polymer alkaline phosphatase red Detection kit (Leica Microsystems) was used to obtain a red reaction product. A variety of procedures were employed to minimize run-to-run variability, including the use of control slides from earlier batches to assess staining penetration. Slides showing evidence of poor penetration were re-stained.

Collecting SMN indices

The number and area of SMNs, were determined. In addition, the area of the ventral horns were determined to obtain the density of SMN/mm^2 of the ventral horn. Computer-assisted microscopy, systematic sampling, and non-biased stereologic object counting methods (Optical dissector; Microbrightfield, Stereoinvestigator Software 8.0, MBF Bioscience, Williston, VT, USA) were used. This was facilitated by the use of a motorized stage (three axis computer-controlled stepping motor system comprised of *XY* stepping stage, *Z*-axis motor and three axis controller with interface to PC).

First, the region of interest (ventral horn of the spinal cord) was delimited at low power, a virtual grid of predetermined size was randomly placed over the entire region by the software program. Magnification was then increased to 200 × and the program engaged to direct the motorized stage on the microscope to stop at each intersection point of the grid for sampling. Sections were systematically studied using the optical dissector and approximately 50–75 sampling sites were documented. The computer design is based on the unbiased bidimensional sampling method originally described by Gunderson and later adapted for three-dimensional counting. Sections were always sampled from the interior at a depth of 10 µm of an area to account for poorly defined borders or trends [44]. The nucleator also used to determine the area of the SMNs. Only SMNs having nuclei were counted.

We employed a component of variance analysis to examine the variance in the SMN measures (SMN counts, area and density) due to persons as compared to the four slides/block which were examined. The major source of variation in these measures of about 80–90% was due to person with the component of variation due to block being negligible (< 1%) and variation due to slides small, ranging from 8 to 13% (Online Resource Table e1). The latter reinforces our preliminary findings that it was sufficient to collect four slides/block.

Determining spinal levels of lumbar cord specimens

Traditional external anatomical landmarks were employed to identify and collect spinal L4–5 lumbar spinal cord segment from which SMN indices were collected. In animal models, the position of motor neuron pools within the spinal cord is conserved and its position dictates the shape of the ventral horn [41]. In recent work, we have extended this observation to humans [26]. The location of the motor neuron column shows a gradual change in its location from the rostral to caudal level of lumbar spinal enlargement. To circumvent differences in spinal cord length among humans and across species, a relative scale was developed which divided the lumbar enlargement into ten segments. The rostral extent of the lumbar enlargement was identified by the most rostral labeled iliopsoas motoneurons and the caudal end was demarcated by the level of labeled pelvic floor motoneurons in Onuf's nucleus. In these prior studies, we validated an approach through which inspection of the shape of the ventral horn in a spinal cord cross-section and comparison to a standardized series could be used to localize spinal cross-sections within the rostral-caudal extent of the lumbar spinal enlargement. The ten levels which can be identified show differential contributions to the innervation of muscle groups.

This approach was not available at the time that SMN indices were collected. Nonetheless, we applied this approach to determine the spinal levels from which SMN indices were collected. Categorization of each spinal tissue block from which spinal motor neuron data was collected was based on the microscopic review of all the slides from each block. A single investigator was blinded to any clinical or postmortem data and categorized the blocks by matching the shape of the ventral horn of each slide with a standardized series validated across several species [26, 41]. More than 80% of the cases were obtained from levels 3, 4 or 5 (Online Resource 1 Table e2). SMN indices were not related to the differences in spinal levels that were studied (Online Resource 1 Table e1). Therefore, based on these analyses, we analyzed all cases together in these analyses.

Assessment of lumbar microglia

Immunohistochemistry for microglia was performed using an Automated Leica Bond immunostainer (Leica Microsystems) and a monoclonal antibody to human HLA-DP, HLA-DQ and HLA-DR (clone CR343, 1:100 dilution, DakoCytomation, Carpinteria, CA, USA) using Bond heat-induced epitope retrieval two solution for 20 min. The Bond Polymer refine detection system (Leica Microsystems) was used which gave a brown reaction product. An investigator blinded to the clinical and pathologic data, outlined the ventral horn area on each slide using the Microbrightfield Stereology System. Using separate tags for stage 1, 2 and 3 microglia, the operator marked the microglia at each intersection point of a 1000 × 750 µm counting frame at 400 × magnification. These counts were then up-weighted by the stereology software to estimate total number of microglia (stage 1, 2 and 3) in the defined area. Different stages of activation from least (stage 1) to most (stage 3) activated were defined morphologically. When microglia become activated, their long fine processes contract and thicken and the cell body adopts a larger more rounded cellular conformation.

Bivariate correlations were used to compare the relationship between SMN indices and other postmortem brain indices and clinical covariates. Two sample *t* tests were used to compare SMN indices between men and women. Separately for men and for women, we used linear regression models to document the relation between SMN and global motor scores proximate to death, controlling for age. We then repeated these models with men and women together and added terms for sex and its interaction with SMN indices to test whether SMN indices and global motor scores varied with sex. In further analyses, we added terms for other postmortem indices and clinical covariates which might attenuate the association of SMN indices with global motor scores.

Data availability

All data included in these analyses are available via the Rush Alzheimer's Disease Center Research Resource Sharing Hub, which can be found at http://www.radc.rush.edu. It has descriptions of the studies and available data. Any qualified investigator can create an account and submit requests for deidentified data.

Results

SMN indices

The clinical characteristics proximate to death and the postmortem indices of the 145 cases are included in Table 1. The average SMN count was 79 (SD 30.2); range 28–168. Average SMN area was 1.8 µm^2 (SD 0.64); range 0.64–3.53 Average SMN count per ventral horn area was 0.4/mm^2 (SD 0.16), range 0.14–1.07.

SMN counts, density and area were not related to age and were similar in both men and women (results not shown). SMN counts were related to SMN area (counts: $r = -0.80$, $p < 0.001$ and SMN density ($r = 0.72$, $p < 0.001$).

Table 1 Clinical and postmortem characteristics ($N = 145$)

Clinical measure	Mean (SD) or *N* (%)
Age at death	89.0 (5.92)
Female sex	99 (68%)
Years of education	14.3 (2.92)
Time from last motor exam to death (years)	1.8 (1.50)
Mini-mental status (0–30)	21.6 (8.58)
Global cognition (proximate to death)	− 0.83 (1.056)
Dementia proximate to death	59 (41%)
Body mass index (kg/m^2)	26.0 (4.79)
Global motor score (proximate to death)	0.77 (0.20)
Vascular diseases	0.8 (1.00)
Claudication	34 (24%)
Congestive heart failure	14 (12%)
Myocardial infarct	32 (22%)
Stroke	31 (22%)
Vascular risk factors	1.3 (0.83)
Hypertension	98 (68%)
Diabetes	32 (22%)
History of smoking	57 (42%)
Postmortem measures	
Postmortem interval (hours)	6.8 (4.32)
Pathologic AD (AD reagan)	80 (55%)
Composite AD pathology	0.6 (0.59)
Tangle density	6.2 (8.50)
Amyloid burden	1.5 (1.14)
Neocortical TDP-43	16 (11%)
Macroinfarcts present	41 (28%)
Microinfarcts present	35 (24%)
Atherosclerosis (mod–sev)	70 (48%)
Arteriolosclerosis (mod–sev)	62 (43%)
Cerebral amyloid angiopathy	9 (6%)
Hippocampal sclerosis (any)	10 (7%)
Lewy bodies (any)	27 (19%)
Nigral neuronal loss (mod–sev)	18 (12%)

SMN and other postmortem indices

While there were several modest correlations between SMN indices and brain pathologies (Table e3), these correlations were no longer significant after we corrected for multiple comparisons.

Recent work has suggested that activated microglia may be associated with loss of SMNs in motor neuron diseases [33]. In a subset of cases analyzed above [$N=38$; age at death 89.0 (SD 5.30); 71% female], stereological sampling in the ventral horn was used to count activated microglia. The mean total number of activated microglia (stages II and III) in the ventral horn was 30.4 (SD 27.3; range 0, 107). In these cases, the mean SMN count was 70 (SD 19.8). As illustrated in Fig. 1, activated spinal microglia counts were inversely related to SMN counts ($r=-0.39$, $p=0.016$).

SMN indices and level of motor function proximate to death

Linear regression models which controlled for age and sex did not show an association between SMN indices and global motor score proximate to death SMN count (Est. 0.016, SE, 0.026, $p=0.548$); SMN density (Est. 0.058, SE, 0.104, $p=0.579$); SMN area (Est. − 0.035, SE 0.032, $p=0.276$). The difference in global motor score between men (0.81, SD 0.22) and women (mean 0.75, SD 0.19) was not significantly different ($t_{136}=-1.67$, $p=0.100$).

While men and women showed similar clinical motor function, the association between SMN indices and motor function might differ. We compared the associations of SMN indices and global motor score in men and women in a single regression model by including terms for age, sex, and an interaction term for SMN indices and sex. The associations of SMN counts and SMN density with global motor score varied by sex (SMN counts × male, Est. 0.174, SE 0.057, $p=0.003$) and (SMN density × male, Est. 0.658, SE 0.233, $p=0.005$).

To further understand this sex interaction, we examined the association of SMN indices and motor function proximate to death in men and women using separate linear regression models controlling for age. A higher SMN count (Est. 0.140, SE 0.052, $p=0.010$) and SMN density (Est. 0.556, SE 0.217, $p=0.014$) were related to better global motor scores proximate to death in men but not women. Inspection of the R^2 in these models in men, suggests that these SMN indices accounted for more than 10% of the variance of motor function proximate to death (SMN counts: 13.5%, SMN density: 12.0%). SMN area was not associated with global motor score in either men (Est. − 0.085, SE 0.619, $p=0.178$) or women (Est. − 0.007, SE 0.038, $p=0.855$).

The model estimates for these associations were similar when we added a term for the time from the last motor exam and death, SMN count (Est. = 0.121, SE 0.059, $p=0.045$) and SMN density (Est. = 0.473, SE 0.252, $p=0.068$). These spinal indices still accounted for an additional 7% (SMN count) and 6% (SMN density) compared to demographics and time from last visit which together accounted for 5% of the variance of global motor score proximate to death.

The associations of SMN counts and density with global motor score in males were not attenuated when controlling

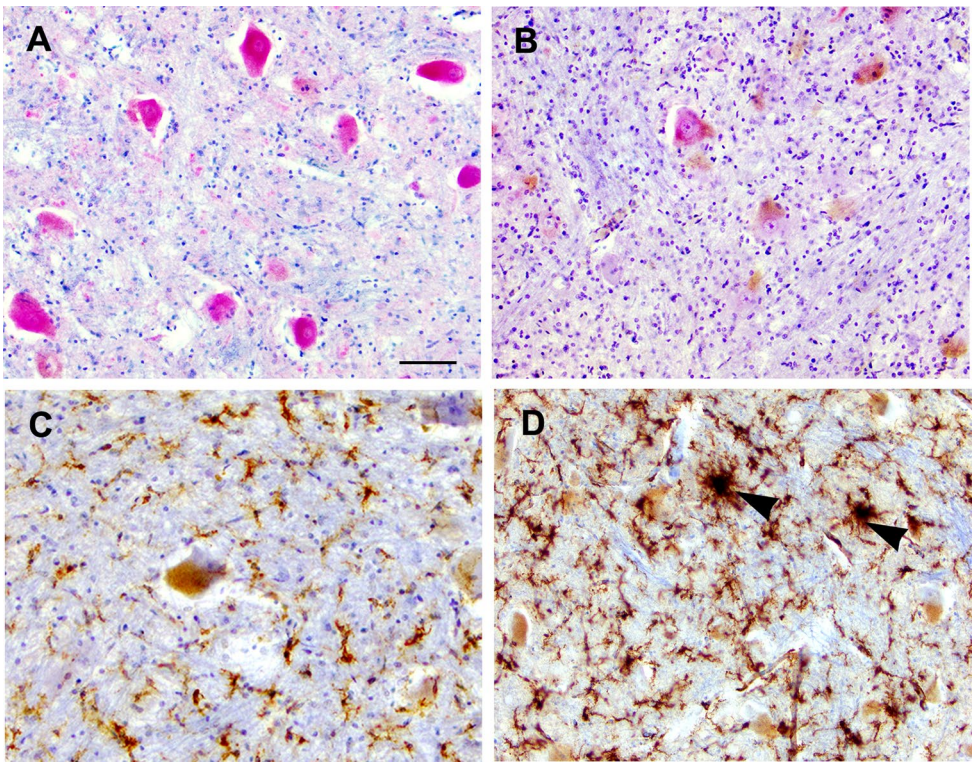

Fig. 1 Number of spinal motor neurons is inversely related to the number of activated microglia. Choline acetyltransferase immunostaining of the ventral horn shows an increased number of spinal motor neurons in **a** as compared to another case **b** which shows a lower number of spinal motor neurons associated with increased nuclear profiles due to an increase in microglia and astroglia. MHC II immunostaining for microglia showed few activated microglia (**c**) associated with the higher neuronal numbers, while the case with decreased number of spinal neurons showed more activated microglia with increased Stage II and III (arrowheads) microglia (**d**). **a**–**d** Scale bar 100 µm

for indices of other brain pathology (Table 2). These models showed similar estimates when we controlled for the time from the last motor assessment and death (Online Resource Table e4).

The associations of SMN indices with global motor score in men were not attenuated when we controlled for body composition, vascular diseases and risk factors and cognitive function (Table 3). These models showed similar estimates when we controlled for the time from the last motor assessment and death (Online Resource Table e5).

Discussion

In this study of older community-dwelling adults, SMN counts and density were associated with motor function proximate to death in men but not women and accounted for more than 10% of the variance of their level of motor function proximate to death. These findings were robust and were not attenuated when we adjusted for other postmortem indices of brain pathology or several clinical covariates. Higher levels of activated microglia were associated with lower counts of spinal motor neurons. Together, these data suggest that loss of spinal motor neuron in older men may contribute in part to late-life motor impairment.

Studies of adults with spinal motor neuron disorders such as ALS or polio underscore the inter-relationship of SMN and motor function [3, 24, 31, 32]. But these conditions are too rare to account for impaired motor function in many older adults. Age-related degeneration of spinal motor neuron has been documented in older adults without overt disease, but clinical measures were lacking [29, 40, 43]. Neurogenic muscle atrophy is a common finding in muscle obtained from living older adults [42]. In contrast to muscle

Table 2 SMN counts and density and motor function in men proximate to death controlling for age and indices of brain pathology

Model	Column A SMN counts and global motor score		Column B SMN density and global motor score	
	Estimate (standard error)	*p* value	Estimate (standard error)	*p* value
1	0.140 (0.052)	0.010	0.555 (0.219)	0.015
2	0.139 (0.052)	0.010	0.550 (0.218)	0.016
3	0.137 (0.053)	0.013	0.549 (0.219)	0.016
4	0.149 (0.050)	0.005	0.618 (0.210)	0.006
5	0.137 (0.051)	0.011	0.531 (0.217)	0.019
6	0.151 (0.050)	0.004	0.567 (0.210)	0.010
7	0.157 (0.052)	0.005	0.620 (0.220)	0.007
8	0.136 (0.053)	0.014	0.540 (0.224)	0.021
9	0.147 (0.055)	0.011	0.567 (0.226)	0.017
10	0.142 (0.054)	0.011	0.574 (0.228)	0.016

A series of regression models were used to examine whether common brain pathologies attenuated the association of SMN counts and SMN density with the outcome of global motor scores in men. Each regression model included age (not shown), SMN counts (Column A) or SMN density (Column B) and indices for one of the following ten brain pathologies measured: (1) AD pathology, (2) Lewy bodies, (3) nigral neuronal loss, (4) TDP-43, (5) hippocampal sclerosis, (6) macroinfarcts, (7) microinfarcts, (8) atherosclerosis, (9) arteriolosclerosis and (10) cerebral amyloid angiopathy

Table 3 SMN indices, clinical covariates and global motor scores in men proximate to death

Model	Column A SMN counts and global motor score		Column B SMN density and global motor score	
	Estimate (standard error)	*p* value	Estimate (standard error)	*p* value
1	0.128 (0.053)	0.020	0.517 (0.220)	0.024
2	0.125 (0.052)	0.020	0.502 (0.215)	0.025
3	0.140 (0.052)	0.011	0.557 (0.221)	0.016
4	0.138 (0.053)	0.012	0.548 (0.223)	0.019

A series of regression models were used to examine whether clinical covariates associated with motor function attenuated the association of SMN counts and SMN density with global motor scores in men. Each regression model included age (not shown). SMN counts (Column A) or SMN density (Column B) and terms for other clinical covariates. Model 1: linear and quadratic terms for BMI (centered at 26); Model 2: global cognition; Model 3: number of three vascular diseases; Model 4: number of our vascular risk factors

fiber degeneration caused by diverse conditions, damage to the peripheral motor nerve or loss of SMN causes characteristic neurogenic muscle atrophy which impairs motor function [21, 34, 35, 39]. However, data about SMN in older adults are lacking since in contrast to muscle, spinal cord specimens cannot be obtained in living older adults.

This study fills several important gaps in knowledge by providing novel data about SMN indices in older community-dwelling adults. First, in contrast to prior smaller autopsy studies, the current study did not find an association between age and SMN indices [19, 30, 40, 43, 47]. It is not clear if this lack of association with age is due to the truncated age range of the adults which were analyzed or whether a larger study is needed. Second, although some animal studies suggest possible sex differences in SMN, in the current study SMN indices were similar in men and women [36]. Third, there was a sex difference in the association of SMN counts and density with level of motor function proximate to death. Prior studies have reported sex differences in muscle fatigue [17]. Prior work has focused on muscle or neuromuscular junction sex differences to account for these behavioral differences [27]. The current study suggests that there may also be sex differences proximal to the neuromuscular junction at the level of the SMN which might contribute to sex differences in fatigue [28].

Movement is a complex volitional behavior whose initiation and execution depend on neural systems that originate in the brain. The neural systems that underlie all movement extend beyond the brain, traversing the brainstem to the spinal cord, to influence spinal motor neurons which regulate skeletal muscle activity, the final effector of all movement. Damage to any portion of the motor system in its course through the CNS can impair motor function in older adults. Brain imaging and autopsy studies suggest that a wide range of age-related brain pathologies accumulate in older brains and their presence is associated with lower levels and more rapid motor decline proximate to death [7, 11, 12, 14, 16]. However, prior studies in this cohort suggest that diverse brain pathologies which accumulate in the older brains account for only a small fraction (< 10%) of the variance of motor function proximate to death [14]. These data suggested that postmortem indices from outside the cerebrum might account for some of this unexplained variance [7, 9, 10].

In the current study, SMN indices alone accounted for more than 10% of the variance of motor function proximate to death. This suggests that SMN indices alone may account for a larger percentage of the variance of motor function than diverse supraspinal brain pathologies. Due to limited power in the current study, we could not compare the contributions of SMN indices and brain pathologies directly in a single model. While these data highlight the importance of SMN and motor function, these data also suggest that it will necessary to examine all of the motor-related regions contributing to motor pathways to determine the pathologic basis for late-life motor impairment. While accumulating evidence underscores complex multifactorial pathologies underlying motor impairment in older adults, it also offers the potential for targeted regional therapies which may halt or slow late-life motor impairment.

The link between SMN indices and motor function in older adults without overt neurologic diseases is unclear. There is a paucity of data about whether age-related neuropathologies known to contribute to neuronal loss in brain also accumulate in the spinal cord of older adults without overt disease or trauma. Recent work in this cohort suggests arteriolosclerosis and Lewy body pathology accumulate in the spinal cord and are related to poorer motor function in older adults [7, 9]. Thus, loss of SMNs in older adults may be due in part to the presence of unrecognized spinal neuropathologies. Accumulating evidence suggests that activated microglia may contribute to ALS, a degenerative disorder characterized by the loss of SMN [33]. The presence of spinal arteriolosclerosis in the current cases in the absence of microinfarcts suggests that age-related loss of spinal motor neurons may be due not to ischemic damage which is common in the cerebrum but to other mechanisms. The association of activated microglia with SMN counts in the current study lends support to the idea that inflammation in the absence of microscopic infarcts might nonetheless contribute to loss of SMN in older adults. Finally, it is possible that other mechanisms not detected in current neuropathologic examination may interfere with SMN function in older adults. While the findings in the current study need to be replicated in other cohorts and in larger studies, these findings provide additional evidence which suggests that degeneration and accumulation of pathology in CNS regions outside the brain may be unrecognized contributors to the development of late-life motor impairment.

Although it well-known that there are anatomic variants that contribute to differences in actual spinal levels, generally spinal specimens are analyzed as deriving from spinal levels based on the conventional landmarks. The current data set provided a unique opportunity to employ an approach recently validated in humans which suggests that the shape of the ventral horn may be a more accurate indicator of the lumbar level of spinal cross-sections in human and other species. These data suggest that more rigorous focus on external anatomical landmarks may not likely improve results. The actual levels obtained can only be determined after fixation of the spinal cord by reviewing the internal landmarks of the spinal cord slides. The heterogeneity of the spinal levels in the current study did not affect our results, but should be considered as a potential source of variability in future human studies of the

spinal cord. It will be crucial to determine the specificity of the spinal levels for the particular phenotypes which is being studied.

There are several limitations to the current study. The cases we studied are from a selected cohort with a restricted age range. This was a small study and may account for the lack of findings in females and underscore the importance of further studies in a larger number of cases in more diverse cohorts. Identifying unmeasured confounding pathologies is always a concern in observational studies. The current study did not assess spinal white matter integrity or other spinal pathologies or crucial motor structures in the PNS.

There are several strengths to the study, including the community-based cohort with both women and men coming to autopsy following high rates of clinical follow-up and high autopsy rates. Postmortem indices of both brain and spinal cord were available on the cases examined in this study. Uniform structured clinical procedures were employed that included a detailed assessment of motor function that has been widely used in other studies.

The current study adds to other recent studies in this cohort that have shown that spinal pathologies are related to poor motor function in older adults [6, 9]. Together these studies provide evidence that not only brain pathologies but also the accumulation of neuropathologies in the spinal cord as well as degeneration of spinal cellular elements may make independent contributions to loss of motor function in older adults without overt neurologic diseases. To translate these findings into the clinical setting, further work is needed to determine the molecular pathways underlying these spinal cord changes and to what degree these mechanisms are region specific. Finally, apart from underscoring the importance of conventional histopathology, these data also suggest that to determine the full extent to which CNS degeneration and pathologies may contribute to late-life motor impairment will require additional studies which quantify postmortem indices in all of the CNS and PNS structures which are crucial for movement.

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Compliance with ethical standards

Conflicts of interest The authors do not declare any conflict of interest for this manuscript.

Ethical standards The study was approved by the Institutional Review Board of Rush University Medical Center.

Informed consent Written informed consent was obtained from all study participants.

References

1. Albers MW, Gilmore GC, Kaye J, Murphy C, Wingfield A, Bennett DA, Boxer AL, Buchman AS, Cruickshanks KJ, Devanand DP, Duffy CJ, Gall CM, Gates GA, Granholm AC, Hensch T, Holtzer R, Hyman BT, Lin FR, McKee AC, Morris JC, Petersen RC, Silbert LC, Struble RG, Trojanowski JQ, Verghese J, Wilson DA, Xu S, Zhang LI (2015) At the interface of sensory and motor dysfunctions and Alzheimer's disease. Alzheimers Dement 11:70–98
2. Ambrose AF, Paul G, Hausdorff JM (2013) Risk factors for falls among older adults: a review of the literature. Maturitas 75:51–61
3. Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW (1991) The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1:103–116
4. Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS (2012) Overview and findings from the rush memory and aging project. Curr Alzheimer Res 9:646–663
5. Buchman AS, Leurgans SE, Boyle PA, Schneider JA, Arnold SE, Bennett DA (2011) Combinations of motor measures more strongly predict adverse health outcomes in old age: the rush memory and aging project, a community-based cohort study. BMC Med 9:42
6. Buchman AS, Leurgans SE, Nag S, VanderHorst V, Kapasi A, Schneider JA, Bennett DA (2017) Spinal arteriolosclerosis is common in older adults and associated with parkinsonism. Stroke 48:2792–2798
7. Buchman AS, Leurgans SE, Nag S, Vanderhorst VGJM, Kapasi A, Schneider JA, Bennett DA (2017) Spinal arteriolosclerosis is common in older adults and associated with parkinsonism. Stroke **(in press)**
8. Buchman AS, Leurgans SE, Weiss A, VanderHorst V, Mirelman A, Dawe R, Barnes LL, Wilson RS, Hausdorff JM, Bennett DA (2014) Associations between quantitative mobility measures derived from components of conventional mobility testing and Parkinsonian gait in older adults. PLoS One 9:e86262
9. Buchman AS, Nag S, Leurgans SE, Miller J, VanderHorst V, Bennett DA, Schneider JA (2017) Spinal Lewy body pathology in older adults without an antemortem diagnosis of Parkinson's disease. Brain Pathol
10. Buchman AS, Nag S, Shulman JM, Lim AS, VanderHorst VG, Leurgans SE, Schneider JA, Bennett DA (2012) Locus coeruleus neuron density and parkinsonism in older adults without Parkinson's disease. Mov Disord 27:1625–1631
11. Buchman AS, Shulman JM, Nag S, Leurgans SE, Arnold SE, Morris MC, Schneider JA, Bennett DA (2012) Nigral pathology and parkinsonian signs in elders without Parkinson disease. Ann Neurol 71:258–266
12. Buchman AS, Wilson RS, Shulman JM, Leurgans SE, Schneider JA, Bennett DA (2016) Parkinsonism in older adults and its association with adverse health outcomes and neuropathology. J Gerontol A Biol Sci Med Sci 71:549–556
13. Buchman AS, Yu L, Boyle PA, Levine SR, Nag S, Schneider JA, Bennett DA (2013) Microvascular brain pathology and late-life motor impairment. Neurology 80:712–718

14. Buchman AS, Yu L, Wilson RS, Boyle PA, Schneider JA, Bennett DA (2014) Brain pathology contributes to simultaneous change in physical frailty and cognition in old age. J Gerontol Ser A Biol Sci Med Sci 69:1536–1544
15. Buchman AS, Yu L, Wilson RS, Schneider JA, Bennett DA (2013) Association of brain pathology with the progression of frailty in older adults. Neurology 80:2055–2061
16. Buchman AS, Yu L, Wison RS, Dawe R, VanderHorst V, Schneider JA, Bennett DA (2015) Post-mortem brain pathology is related to declining respiratory function in community-dwelling older adults. Front Aging Neurosci 7
17. Clark BC, Collier SR, Manini TM, Ploutz-Snyder LL (2005) Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. Eur J Appl Physiol 94:196–206
18. Corcos DM, Chen CM, Quinn NP, McAuley J, Rothwell JC (1996) Strength in Parkinson's disease: relationship to rate of force generation and clinical status. Ann Neurol 39:79–88
19. Cruz-Sanchez FF, Moral A, Tolosa E, de Belleroche J, Rossi ML (1998) Evaluation of neuronal loss, astrocytosis and abnormalities of cytoskeletal components of large motor neurons in the human anterior horn in aging. J Neural Transm (Vienna, Austria: 1996) 105:689–701
20. Dawe RJ, Yu L, Leurgans SE, Schneider JA, Buchman AS, Arfanakis K, Bennett DA, Boyle PA (2016) Postmortem MRI: a novel window into the neurobiology of late life cognitive decline. Neurobiol Aging 45:169–177
21. Doherty TJ (2003) Invited review: aging and sarcopenia. J Appl Physiol 95:1717–1727
22. Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, Guralnik JM (2000) Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. J Am Geriatr Soc 48:1618–1625
23. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56:M146–M156
24. Giannakopoulos P, Hof PR, Michel JP, Guimon J, Bouras C (1997) Cerebral cortex pathology in aging and Alzheimer's disease: a quantitative survey of large hospital-based geriatric and psychiatric cohorts. Brain Res Brain Res Rev 25:217–245
25. Gosker HR, Wouters EF, van der Vusse GJ, Schols AM (2000) Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. Am J Clin Nutr 71:1033–1047
26. Gross C, Ellison B, Buchman AS, Terasawa E, VanderHorst VG (2017) A novel approach for assigning levels to monkey and human lumbosacral spinal cord based on ventral horn morphology. PLoS One 12:e0177243
27. Hunter SK (2016) Sex differences in fatigability of dynamic contractions. Exp Physiol 101:250–255
28. Johnson ST, Kipp K, Hoffman MA (2012) Spinal motor control differences between the sexes. Eur J Appl Physiol 112:3859–3864
29. Kawamura Y, Okazaki H, O'brien PC, Dyck PJ (1977) Lumbar motoneurons of man: I) Number and diameter histogram of alpha and gamma axons of ventral root. J Neuropathol Exp Neurol 36:851–860
30. Kawamura Y, Okazaki H, O'brien PC, Dyck PJ (1977) Lumbar motoneurons of man: II) The number and diameter distribution of large and intermediate-diameter cytons in "motoneuron columns" of spinal cord of man. J Neuropathol Exp Neurol 36:861–870
31. Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, Oikonen V, Kailajarvi M, Scheinin M, Viitanen M, Parkkola R, Rinne JO (2007) PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. Neurology 68:1603–1606
32. Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, Oikonen V, Kailajarvi M, Scheinin M, Viitanen M, Parkkola R, Rinne JO (2006) Voxel-based analysis of PET amyloid ligand [11C]PIB uptake in Alzheimer disease. Neurology 67:1575–1580
33. Lee J, Hyeon SJ, Im H, Ryu H, Kim Y, Ryu H (2016) Astrocytes and microglia as non-cell autonomous players in the pathogenesis of ALS. Exp Neurobiol 25:233–240
34. Lexell J, Downham DY (1991) The occurrence of fibre-type grouping in healthy human muscle: a quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years. Acta Neuropathol (Berl) 81:377–381
35. Ling SM, Conwit RA, Ferrucci L, Metter EJ (2009) Age-associated changes in motor unit physiology: observations from the Baltimore Longitudinal Study of Aging. Arch Phys Med Rehabil 90:1237–1240
36. Mierzejewska-Krzyzowska B, Bukowska D, Taborowska M, Celichowski J (2014) Sex differences in the number and size of motoneurons innervating rat medial gastrocnemius muscle. Anat Histol Embryol 43:182–189
37. Rosso AL, Studenski SA, Chen WG, Aizenstein HJ, Alexander NB, Bennett DA, Black SE, Camicioli R, Carlson MC, Ferrucci L, Guralnik JM, Hausdorff JM, Kaye J, Launer LJ, Lipsitz LA, Verghese J, Rosano C (2013) Aging, the central nervous system, and mobility. J Gerontol Ser A Biol Sci Med Sci 68:1379–1386
38. Rowland LP (1998) Diagnosis of amyotrophic lateral sclerosis. J Neurol Sci 160(Suppl 1):S6–S24
39. Stalberg E, Fawcett PR (1982) Macro EMG in healthy subjects of different ages. J Neurol Neurosurg Psychiatry 45:870–878
40. Tomlinson BE, Irving D (1977) The loss of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci 34:213–219
41. Vanderhorst VG, Holstege G (1997) Organization of lumbosacral motoneuronal cell groups innervating hindlimb, pelvic floor, and axial muscles in the cat. J Comp Neurol 382:46–76
42. Vandervoort AA (2002) Aging of the human neuromuscular system. Muscle Nerve 25:17–25
43. Wang Y, Hashizume Y, Yoshida M, Inagaki T, Kameyama T (1999) Pathological changes of the spinal cord in centenarians. Pathol Int 49:118–124
44. Williams RW, Rakic P (1988) Three-dimensional counting: an accurate and direct method to estimate numbers of cells in sectioned material. J Comp Neurol 278:344–352
45. Wilson RS, Segawa E, Buchman AS, Boyle PA, Hizel LP, Bennett DA (2012) Terminal decline in motor function. Psych Aging 4:988–1007
46. Yogev-Seligmann G, Giladi N, Gruendlinger L, Hausdorff JM (2013) The contribution of postural control and bilateral coordination to the impact of dual tasking on gait. Exp Brain Res 226:81–93
47. Zhang C, Goto N, Suzuki M, Ke M (1996) Age-related reductions in number and size of anterior horn cells at C6 level of the human spinal cord. Okajimas Folia Anat Jpn 73:171–177