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Research paper

Age as a determinant of inflammatory response and survival of glia and axons after human traumatic spinal cord injury

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ABSTRACT

Despite the shift in the demographics of traumatic spinal cord injury (SCI) with increased proportion of injuries in the elderly, little is known on the potential effects of old age on the pathobiology of SCI. Since there is an assumption that age adversely affects neural response to SCI, this study examines the clinically relevant question on whether age is a key determinant of inflammatory response, oligodendroglial apoptosis and axonal survival after traumatic SCI. This unique study includes post-mortem spinal cord tissue from 64 cases of SCI (at cervical or high-thoracic levels) and 38 control cases without CNS injury. Each group was subdivided into subgroups of younger and elderly individuals (65 years of age or older at the SCI onset). The results of this study indicate that age at the SCI onset does not adversely affect the cellular inflammatory response to, oligodendroglial apoptosis and axonal survival after SCI. These results support the conclusion that elderly individuals have similar neurobiological responses to SCI as younger people and, hence, treatment decisions should be based on an assessment of the individual patient and not an arbitrary assumption that "advanced age" should exclude patients with an acute SCI from access to advanced care and translational therapies.

1. Introduction

Traumatic spinal cord injury (SCI) is a potentially catastrophic event for individuals and their family members, with major medical, social and financial implications for the individuals and society. The incidence rates of traumatic SCI vary from 6.2 to 174 per million inhabitants yearly, and differ substantially among countries and continents (Furlan and Tator, 2012). Traumatic SCI is more common among young male adults due to motor vehicle accidents; however, there has been an escalation of fall-related SCI in the elderly as a result of the aging of the global population (Furlan and Tator, 2012). In the United States, the mean age of individuals with traumatic SCI raised from 28.7 years in 1972–1979 to 43.1 years in 2015–2019 (NSCISC, 2019). The proportion of older individuals (65 years or older at the time of injury) increased from 5.6% in 2004 to 7.4% in 2019 in the United States (NSCISC, 2004, 2019).

Aging results in a progressive reduction of reserves in most physiologic systems and an increasing susceptibility to most diseases and to death. The literature on normal aging and immunology supports the notion of a process of peripheral "immunosenescence" involving both adaptive and innate immune systems (Di Benedetto et al., 2017). Franchesi et al. named "inflammaging" a subclinical chronic inflammatory process related to macrophage activation and inflammatory monocytes during aging process (Franceschi et al., 2000; Franceschi et al., 2007). Those age-related changes in the peripheral immune system status were associated with a gradual neuroinflammation process within the neurologically-intact aged brain, which is characterized by augmented glial activation, increased inflammatory cytokines, and reduced anti-inflammatory response in the brain (Di Benedetto et al., 2017). While pathologic neuroinflammatory process has been

Abbreviations: SCI, spinal cord injury; CNS, central nervous system; PBS, phosphate-buffered saline; MMP, matrix metalloproteinase; NF, neurofilament; NASCIS, National Spinal Cord Injury Study; IL, interleukin

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implicated in neurogenerative diseases of the brain, there is a paucity of studies focused on the impact of age at the time of injury on the pathobiology of traumatic SCI. Given this, an improved understanding of the consequences of older age on the neurobiological response to SCI is required. The current understanding of CNS pathophysiology including SCI mostly relies on extrapolations from experimental studies using animal models (Krassioukov and Weaver, 1996). Nonetheless, translational research is crucial in order to confirm that human and animal CNS undergoes similar changes in normal and diseased conditions before the information from animal models can be extrapolated to our knowledge on human CNS pathophysiology. Further investigations on human pathobiology of SCI are recommended due to the paucity of studies focused on histopathological changes within the human spinal cord and, more importantly, because some histopathological findings in humans are significantly different from those observed in animal models of SCI (Hayes and Kakulas, 1997; Puckett et al., 1997).

With this background, a histopathological and immunohistochemical examination of postmortem spinal cord tissue was undertaken to evaluate whether age at the time of injury is a key determinant for cellular inflammatory response to, oligodendroglial apoptosis, and axonal survival after acute traumatic SCI. By determining the role of age/aging in the pathobiology of SCI, this study also provides further insights on the pathobiology that are important for the development of clinical protocols and guidelines for maximizing neurological and functional recovery of individuals with acute SCI according to their age.

2. Material and methods

2.1. Human ethics statement

The research protocol for this study was approved by the Research Ethics Board, University Health Network (Toronto, Canada) and by the Institutional Ethics Board, University of Miami (Miami, USA).

2.2. Post-mortem human spinal cord tissue

This histopathological and immunohistochemical examination of postmortem human spinal cord tissue included 102 cases from the Toronto Western Hospital Spinal Cord Tissue Bank and Miami Project Spinal Cord Tissue Bank. There are 64 cases of SCI (at cervical or high-thoracic levels, T6 or above) and 38 control cases without history of CNS trauma that were matched for age, sex, and spinal level with the cases of SCI. Each group was subdivided into subgroups according the individuals' age at the SCI onset or at time of death in control cases as follows: younger individuals (age range from 16 to 64 years), and elderly individuals (65 years of age or older). Data were analyzed separately in each subgroup of cases of SCI as classified into: acute (up to 30 days), subacute (31 days to 6 months), and chronic (more than 6 months) stages following SCI. Cases were excluded if there was a remote medical history of diabetes mellitus, CNS disease or injury, or CNS surgery that could affect the study results. Given that concurrent systemic inflammation/infection at the time of death could adversely affect the examination of inflammatory response within the post-mortem spinal cord tissue, cases with a clinical history of significant systemic infectious and inflammatory complications not directly related to trauma were also excluded (Lemstra et al., 2007).

2.3. Histology and immunohistochemistry

The sections used for immunohistochemistry were dried, deparaffinized in xylene, and rehydrated through a series of graded ethyl alcohols. Endogenous peroxidase was inactivated by treatment with 3% H2O2 in 100% methanol for 5 min. Following a 5-min wash in phosphate-buffered saline (PBS), the sections underwent microwave heat-induced antigen retrieval in 10 mmol/l citrate buffer, pH 6.0. Non-specific staining was blocked by incubating the sections in 10% normal horse serum for 1 h at room temperature (Shi et al., 2001; Kahveci et al., 2003). Subsequently, the sections were incubated with the following primary antibodies at 4 °C for overnight in 2% normal horse serum in a humidified chamber. Alternate sections (5 to 7 μm) of spinal cord were then immunostained with antibody against: the matrix metalloproteinase 9 (anti- MMP-9; 1:1000, Chemicon International, Temecula, CA, USA), labeling inactive and activated forms of MMP-9+ neutrophils (Noble et al., 2002); anti-CD68 (1:100, Dako, Glostrup, Denmark), a lysosomal protein expressed by phagocytic macrophages of microglial and monocytic origin (Ramprasad et al., 1996; Deininger et al., 2001; Caffo et al., 2005); anti-CD8α (1:100, Dako, Glostrup, Denmark), labeling cytotoxic T and natural killer cells; anti-CD4 (1:100, Novocastra Laboratories Ltd., Newcastle, UK), labeling helper/regulator T cells; anti-CD20cy (1:100, Dako, Glostrup, Denmark), labeling B cells; anti-cleaved caspase-3 (1:200; Cell Signaling Technology, Danvers, MA) labeling apoptotic cells (Staines et al., 1988; Charriaut-Marlangue and Ben-Ari, 1995); anti-APC (1:50; EMD Millipore, Billerica, MA USA), labeling oligodendrocyte cell bodies (CC1); and anti-neurofilament 200, NF 200 (1:100, Sigma-Aldrich Corp., St. Louis, MO, USA). Sections were then washed in PBS and incubated overnight in a donkey anti-mouse secondary antibody conjugated to biotin (1:1000, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) in 2% normal horse serum. Next, sections were washed in PBS and incubated with Vectastain AB (ABC Elite Kit, Vector Laboratories, Burlingame, ON, Canada) in PBS according to manufacturer instructions for 30 min. Tissues were then incubated for 10 min with the NovaRED substrate kit (Vector Laboratories) for visualization of antibody binding. Following PBS washes, the slides were dehydrated through an alcohol series, cleared in xylene, and cover slipped.

2.4. Cell/axon counting and area measurement

Sections were examined using confocal microscope (Nikon Confocal D-Eclipse Microscope). Unbiased stereological techniques were used to minimize the potential pitfalls related to sampling error and double counting of cells in the spinal cord sections immunostained for neutrophils, activated macrophage and apoptosis (Nashmi and Fehlings, 2001; Tandrup, 2004). MMP-9+ neutrophils, activated (CD68+) macrophages, infiltrated lymphocytes (subdivided into CD4+ T cells, CD8+ T cells and CD20cy + B cells), apoptotic cells (Caspase-3+ cells) with morphology of oligodendrocytes and the number of oligodendrocytes (CC1+ cells) within the spinal cord white matter at alternate sections two or three segments caudal to the injury site (or in matched sections at cervical and high thoracic levels from control cases) were manually counted using Image-Pro imaging software. In each area of interest, the cell count was carried out in 5 different fields that were randomly selected avoiding overlapping the fields.

The number of preserved axons within the spinal cord white matter were stained for NF 200 at alternate sections two or three segments caudal to the injury site (or in sections at cervical and high thoracic levels from control cases). The number of axons and the number of cells were manually counted, using Image-Pro imaging software, in 5 different fields from the areas of lateral corticospinal tracts, posterior column, and either anterior corticospinal tracts or descending vasomotor pathways (dorsolateral aspect) in each side of the spinal cord. Axon and cell counting are expressed by mean per 10,000 square microns.

Stained inflammatory cells (i.e. neutrophils, activated macrophages, and infiltrated lymphocytes) were counted within the lateral and anterior corticospinal tracts, and posterior column in order to examine the extend of the inflammatory response across lateral, anterior and posterior areas of the spinal cord white matter. Otherwise, the number of preserved axons and apoptotic cells with morphology of oligodendrocytes and the number of oligodendrocytes were counted within lateral corticospinal tracts, descending vasomotor pathways and

posterior column. Disruption of those white matter tracts has a clinical significance due to motor, autonomic and sensory impairments that are commonly observed in individuals after SCI. The selection of the motor, autonomic and sensory tracts also allowed us to compare the study results with data from a prior histopathological study that analyzed the association of age with axonal preservation and extend of demyelination below the level of SCI (Furlan et al., 2010). Of note, the descending vasomotor pathways were previously found to be located almost adjacent to the lateral corticospinal tracts (Furlan et al., 2003).

2.5. Data analyses

In a prior pilot study, the number of preserved axons within the dorsal column two segments caudal to the injury site was 125 and 98 in elderly and younger SCI individuals, respectively (Furlan et al., 2010). In controls, the number of preserved axons within the dorsal column at low cervical level was 125 and 153 in elderly and younger uninjured individuals, respectively. Using two-tailed Mann Whitney *U* test, the type-II error was estimated to be 7% for the SCI group and 20% for the control group. The comparisons between younger and elderly individuals in each group and subgroup were carried out using two-tailed, Mann-Whitney U test (or Mann Whitney Rank Sum test for non-parametric data) or Fisher exact test. Furthermore, a series multiple regression analyses was performed to evaluate the robustness of the results of the univariate analysis when adjusting data analyses for major potential confounders such as time from the SCI onset to death, individuals' sex, and level and cause of SCI. Because data on the severity of SCI were unavailable in most of the cases in our cohort, the cause of injury was used as an approximate surrogate for the severity of injury in our regression models. Significance level for all tests was set at $p < .05$. All data analyses were performed using SAS program version 8.02 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Baseline data

There were 29 women and 73 men with a mean age of 58.6 years (age range from 16 to 90 years). Of the 102 cases, 39 individuals were elderly who died in the acute ($n = 21$), subacute ($n = 10$) or chronic stage following traumatic SCI ($n = 8$); and there were 17 elderly individuals in the control group. In addition, there were 25 younger individuals who died in the acute ($n = 15$), subacute ($n = 4$) or chronic stage after traumatic SCI ($n = 6$); and 21 younger individuals were included in the control group.

The group of younger individuals with SCI was statistically similar to the group of elderly individuals with SCI with respect to their level of SCI, but the latter group had a significantly higher frequency of fall-related SCIs and lower frequency of SCIs due to other causes than the former group (Table 1). Furthermore, there was a trend towards a greater proportion of females in the group of younger individuals with SCI when compared to their counterparts (Table 1). There were no statistically significant differences between the group of younger individuals with SCI and the group of elderly individuals with SCI in terms of the mean period of time from the SCI onset to death in the subacute SCI subgroup as well as in the chronic SCI subgroup (Table 1). Nonetheless, there was a trend towards a shorter period of time from the SCI onset to death among elderly individuals when compared with younger individuals in the acute SCI subgroup (Table 1). The female-male ratio in the group of younger individuals (ratio of 11:12) did not differ statistically from the group of elderly individuals (ratio of 1:2) among control cases ($p = .546$).

3.2. Data on cell and axon count

Examination of the inflammatory response to SCI indicates that younger and elderly individuals had statistically similar number of infiltrated neutrophils (Fig. 1A and B), number of activated macrophages (Fig. 1C and D), and number of infiltrated lymphocytes (Fig. 2A–F) within the lateral and anterior corticospinal tracts, and posterior column in most of the stages after SCI. However, younger individuals showed significantly greater number of neutrophils within the anterior corticospinal tracts than elderly individuals in the chronic stage after SCI ($p = .049$; Fig. 1B). Also, there was a significantly greater number of activated macrophages within the posterior column in the group of younger individuals when compared with the group of elderly individuals in the acute stage after SCI ($p = .016$; Fig. 1D). Furthermore, there was a trend towards a higher density of infiltrated B-cell lymphocytes within the lateral costicospinal tracts among the younger individuals when compared with elderly individuals in the subacute stage following SCI ($p = .059$; Fig. 2B). In contrast, the group of elderly individuals had a greater density of cytotoxic T and natural killer cells (CD8+ cells) within the lateral corticospinal tracts than their younger counterparts in the subacute stage after SCI ($p = .049$; Fig. 2D).

Using multiple regression analyses, the number of infiltrated neutrophils, number of activated macrophages, and number of infiltrated lymphocytes were not associated with the individual's age at the SCI onset when the models were adjusted for sex, time from the injury to death, and level and cause of SCI (Table 2).

Examination of the number of oligodendroglial apoptosis indicate that younger and elderly individuals had statistically similar number of caspase-3+ cells with morphology of oligodendrocytes (Fig. 3A and B) and similar proportion of Caspase-3+/CC1+ cells (Fig. 3C and D) within the lateral corticospinal tracts, descending vasomotor pathways, and posterior column in all stages after SCI. Using multiple regression analyses, the number of caspase-3+ cells with morphology of oligodendrocytes and the proportion of Caspase-3+/CC1+ cells were not significantly associated with the individual's age at the SCI onset when the models were adjusted for sex, time from the injury to death, and level and cause of SCI (Table 2).

The number of preserved axons within the lateral corticospinal tracts, descending vasomotor pathways, and posterior column did not significantly differ between the group of younger individuals and the group of elderly individuals with SCI or without CNS injury (Fig. 4C).

4. Discussion

The results of the univariate analyses in this study indicate that younger and elderly individuals had similar inflammatory response to SCI with respect to the number of infiltrated neutrophils, activated macrophages, and infiltrated lymphocytes within the spinal cord white matter in most of the stages following SCI. While younger individuals sporadically showed greater inflammatory response (e.g. neutrophils, activated macrophages and infiltrated B-cell lymphocytes) than elderly individuals, the latter had a higher T-lymphocyte mediated response during subacute stage after SCI than the former group. Nonetheless, the results of the multiple regression analyses suggested that there is no age-related effect on the inflammatory response to SCI after adjusting the models for sex, time from the SCI onset to death, and level and cause of SCI. Additionally, the study results suggest that younger and elderly individuals had similar number of oligodendrocytes in apoptosis within the motor, autonomic, and sensory spinal cord tracts in all stages after traumatic SCI when using univariate and multiple regression analyses adjusted for major potential confounders. Finally, younger and elderly individuals did not differ with regards to axonal preservation within the spinal cord white matter following traumatic SCI. Of note, there were no significant differences between the group of younger individuals and the group of elderly individuals without CNS trauma with regards to the number of axons within motor, autonomic and sensory areas of the spinal cord white matter.

Table 1
Baseline data comparing the group of younger individuals with spinal cord injury (SCI) to the group of elderly individuals with SCI.

Features	Elderly individuals with SCI (n = 39)	Younger individuals with SCI (n = 25)	p value
Mean age ± SEM	76.2 ± 1.3 years	38.3 ± 3.0 years	
Age range	65 to 90 years	16 to 64 years	
Sex: n (%)			.067
Females	3 (7.7%)	6 (24.0%)	
Males	36 (92.3%)	19 (76.0%)	
Level of SCI: n (%)			.129
Tetraplegia	34 (87.2%)	18 (72.0%)	
Paraplegia	5 (12.8%)	7 (28.0%)	
Cause of SCI: n (%)			.002
MVA	15 (38.5%)	9 (36.0%)	
Fall	18 (46.1%)	3 (12.0%)	
Other causes	6 (15.4%)	13 (52.0%)	
Mean time of SCI onset to death (± SEM):			
Acute SCI subgroup	7.2 ± 1.6 days	13.1 ± 2.6 days	.097
Subacute SCI subgroup	59.60 ± 12.3 days	62.3 ± 15.9 days	.778
Chronic SCI subgroup	3585.9 ± 1713.2 days	1834.2 ± 1388.5 days	.570

SEM: standard error of mean; MVA: motor vehicle accident; Other causes include violence, gunshot wound, sports, and leisure.

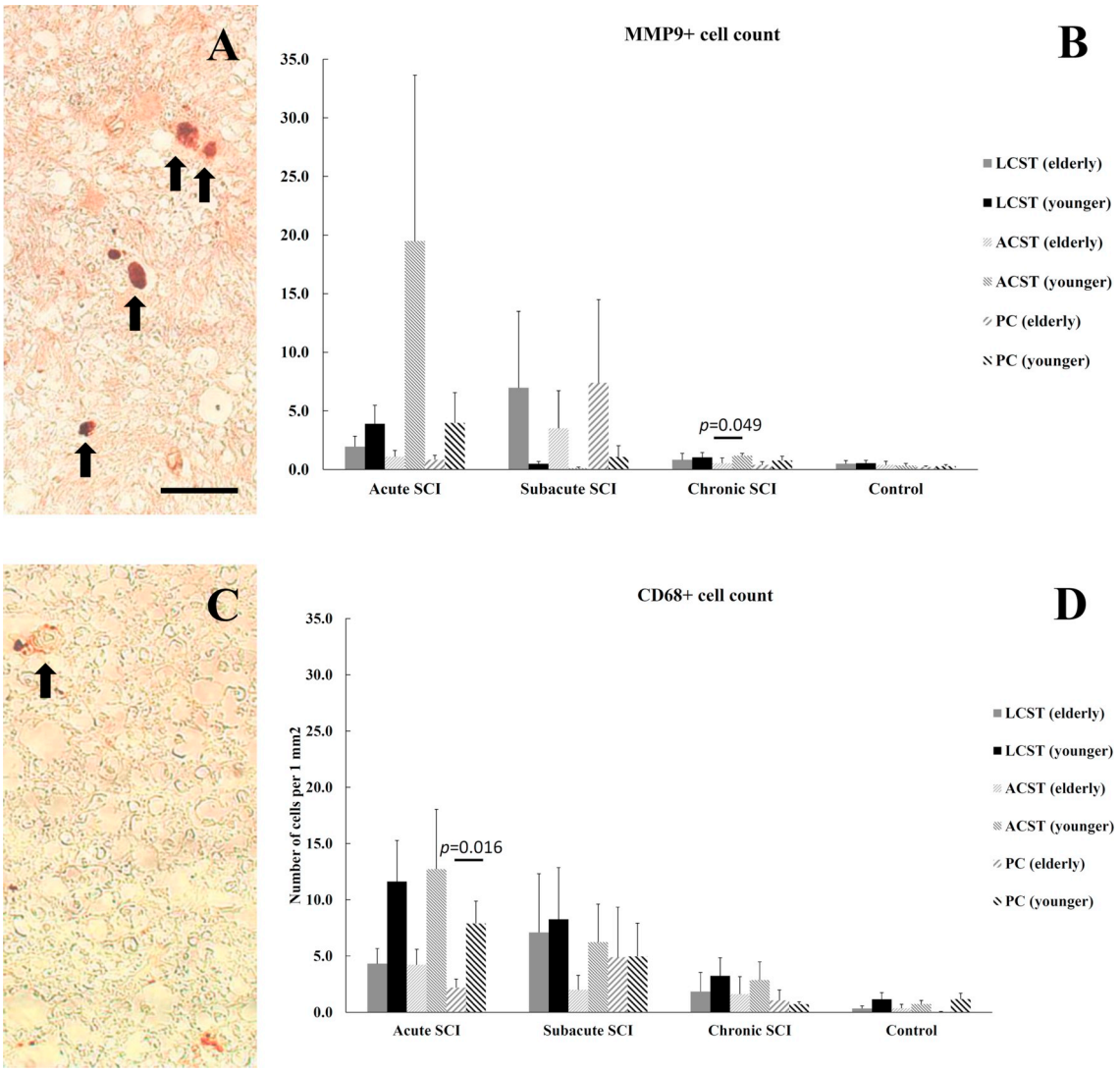
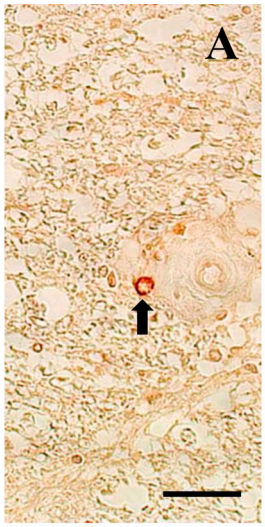
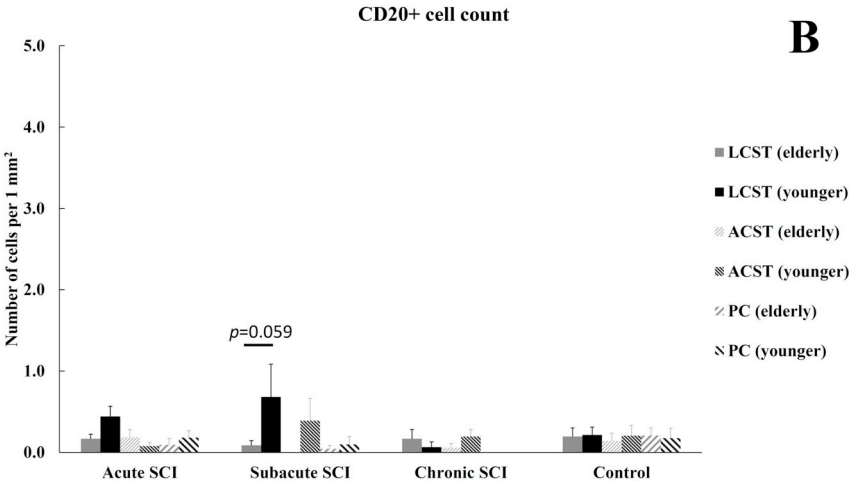
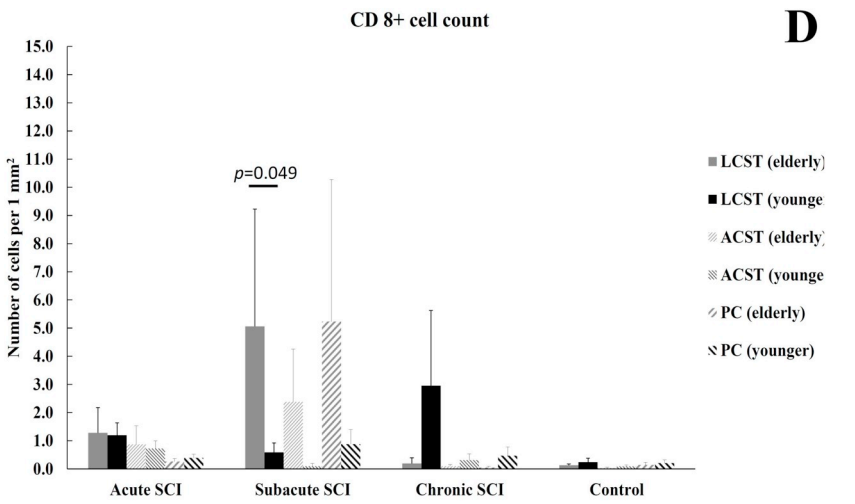
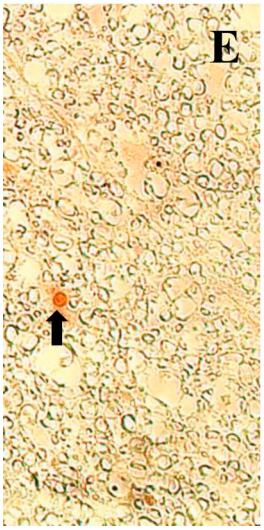


Fig. 1. Photomicrographs of representative areas of spinal cord white matter from a 67-year old male who suffered a severe C2 spinal cord injury (SCI) after fall and died 3.5 months after trauma in high magnification. The mid-cervical section was immunostained for infiltrated neutrophils using MMP-9 immunostaining as indicated by arrows (magnification: x40) (A) and activated macrophages using CD 68 immunostating indicated by arrow (x20) (C). Calibration bar is 50 μm. Results of comparisons between younger group and elderly group with regards to the number of infiltrated neutrophils (B), and activated macrophages (D) within the lateral corticospinal tracts (LCST), anterior corticospinal tracts (ACST) and posterior column (PC) of spinal cord white matter in the acute, subacute and chronic stages following SCI as well as in control cases (most of the paired comparisons had a $p > .05$). Data are represented as mean ± SEM.









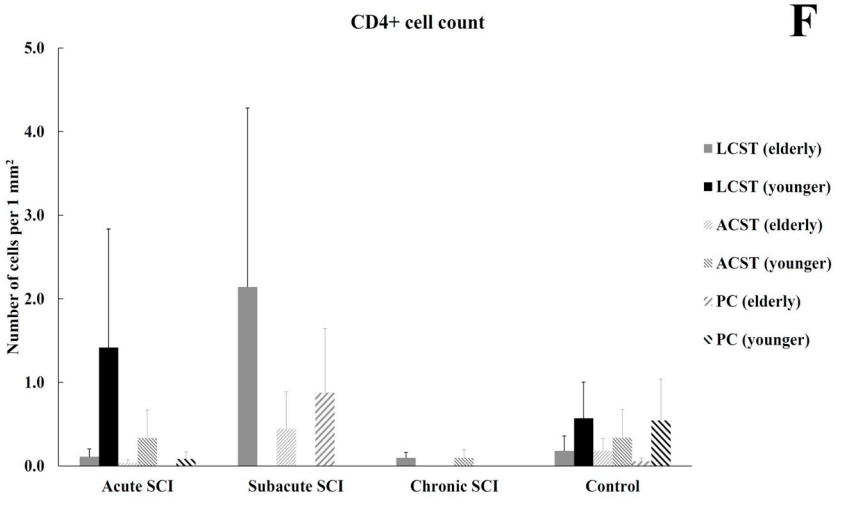


Fig. 2. Photomicrographs of representative areas of spinal cord white matter from a 67-year old male who suffered a severe C2 spinal cord injury (SCI) after fall and died 3.5 months post-trauma in high magnification (x40). The mid-cervical section was immunostained for infiltrated B cells lymphocyte (CD 20+ cell indicated by an arrow) (A), infiltrated cytotoxic T and natural killer cell (CD 8+ cells indicated by an arrow) (C), and infiltrated helper/regulator T cells (CD 4+ cells indicated by arrows) (E). Calibration bar is 50 μm. Results of comparisons between younger and elderly individuals with regard to the number of B cells lymphocytes (B), cytotoxic T and natural killer cell (D), and helper/regulator T cells (F) within the lateral corticospinal tracts (LCST), anterior corticospinal tracts (ACST) and posterior column (PC) of spinal cord white matter in the acute, subacute and chronic stages following SCI as well as in control cases (most of the paired comparisons had a $p > .05$). Data are represented as mean ± SEM.

Table 2
Results of the multiple regression analyses testing the association between cell count and individuals' age at the spinal cord injury (SCI) onset after adjusting for sex (reference: males), time from injury to death, level of SCI (reference: thoracic level), and cause of injury (i.e. reference: other causes).

Dependent variable	R-square	F value	*p* value
CD20+ cell count within the LCST	0.195	1.87	.094
CD20+ cell count within the ACST	0.066	0.54	.797
CD20+ cell count within the PC	0.035	0.28	.960
CD8+ cell count within the LCST	0.109	0.94	.482
CD8+ cell count within the ACST	0.086	0.72	.653
CD8+ cell count within the PC	0.048	0.39	.907
CD4+ cell count within the LCST	0.086	0.68	.685
CD4+ cell count within the ACST	0.134	1.11	.372
CD4+ cell count within the PC	0.037	0.28	.960
CD68+ cell count within the LCST	0.113	0.99	.451
CD68+ cell count within the ACST	0.133	1.17	.338
CD68+ cell count within the PC	0.098	0.84	.560
MMP9+ cell count within the LCST	0.066	0.55	.790
MMP9+ cell count within the ACST	0.255	2.69	.018*
MMP9+ cell count within the PC	0.027	0.22	.980
Caspase 3+ oligodendrocyte count within the LCST	0.130	0.85	.551
Caspase 3+ oligodendrocyte count within the DVP	0.098	0.62	.736
Caspase 3+ oligodendrocyte count within the PC	0.049	0.30	.951
Caspase 3+/CC1+ cell count within the LCST	0.084	0.50	.828
Caspase 3+/CC1+ cell count within the DVP	0.151	0.97	.468
Caspase 3+/CC1+ cell count within the PC	0.044	0.25	.967

* Only the level of SCI was significantly associated with the dependent variable (F value = 6.71; $p = .003$).

4.1. Aging and neuroinflammatory response to traumatic spinal cord injury

Cellular inflammatory response after human SCI is similar to those observed in experimental studies using SCI models in rodents (Fleming et al., 2006). Neutrophils and microglia/macrophages, which are the first cells to participate in the inflammatory response, can release a variety of oxidative and proteolytic enzymes that are involved in secondary injury by extending the lesion and putatively aggravating neurological dysfunction (Fleming et al., 2006). Neutrophils are the first hematogeneous inflammatory cells to arrive at the injury site after SCI having been observed in areas of hemorrhage as early as 4 h in humans after SCI (Fleming et al., 2006). Neutrophils reach a maximum density at 1 to 3 days after injury and remain elevated up to 10 days following SCI (Fleming et al., 2006). Extravascular neutrophils were found in areas of hemorrhage, necrosis and tissue fragmentation in humans (Fleming et al., 2006). Abnormal vascular permeability and tissue damage were found to be commonly associated with MMP expression, more specifically MMP-9, which degrades components of the basal lamina of the blood-CNS barrier (Mun-Bryce and Rosenberg, 1998). Similarly, activated microglia were observed in the lesion by 1 day following human SCI and, reportedly, reached a peak between 1 and 3 days post-injury (Fleming et al., 2006). Activated microglia and macrophages were documented as the predominant inflammatory cells at and beyond 5 to 10 day interval after human SCI (Fleming et al., 2006). In addition to the local neuro-inflammation, SCI reportedly evokes a systemic inflammatory response that causes tissue damage outside of CNS. In a retrospective cohort study, patients with acute SCI showed significant differences in the white cell counts (i.e. leukocytosis and lymphopenia) within the first week post-trauma in comparison with individuals who had spine trauma without neurological deficit (Furlan et al., 2006). Using an animal SCI model, a prior study showed that lungs and kidneys are substantially infiltrated by neutrophils between 2 h and 7 days after injury (Gris et al., 2008). This may be one of the reasons why renal and respiratory failure remains leading causes of death after traumatic SCI (DeVivo et al., 1999; Catz et al., 2002; O'Connor, 2005).

In addition to an immediate and substantial inflammatory response to an abrupt CNS insult, the current literature underlines a chronic low-grade inflammatory response and immune impairment that are typically observed in individuals following traumatic SCI (Allison and Ditor, 2015). Multiple potential causes are involved in a persistent inflammatory state after SCI, including: bidirectional communication between the nervous, endocrine and immune systems; damage to the autonomic nervous system that may induce immune dysfunction, either by loss of neural innervation of lymphoid organs or endocrinal impairment; and secondary health complications and metabolic disorders due to motor, sensory and autonomic impairments following SCI (Allison and Ditor, 2015). Interestingly, the present study also identified a modest degree of infiltrated inflammatory cells within the spinal cord in individuals with chronic SCI even after years from the primary spinal injury.

Biologically, failure of adaptive immune system with aging is recognized as a major cause of morbidity and mortality in the elderly (Grubeck-Loebenstein and Wick, 2002; Weng, 2006; Iancu et al., 2008; Yung and Julius, 2008). For example, a prior study using gene expression microarray and flow cytometry analyses, Czesnikiewicz-Guzik et al. documented that CD8+ T cells are more age sensitive, but CD4+ T cells are less susceptible to aging (Czesnikiewicz-Guzik et al., 2008). While aging was reportedly associated with morphological abnormalities of spinal cord and brain angioarchitecture, the potential influence of age on the blood-brain barrier integrity and neutrophil recruitment in humans after SCI has not been documented to date (Moody et al., 1991; Qiu and Zhu, 2004; Hughes et al., 2006). Studying the effects of sepsis on the activation of microglia, Lemstra et al. found that age did not significantly affect the CD68 immunoreactivity of microglia in postmortem spinal cord sections of individuals who died due to sepsis (Lemstra et al., 2007). In contrast, numerous studies of aging-related immunophenotypic changes in microglia in humans and animal models demonstrated a steady increase in the expression of markers, that are usually upregulated, on activated microglia after acute CNS injuries (Sloane et al., 1999; Streit, 2005; Streit et al., 2005; Conde and Streit, 2006). Nonetheless, the potential effects of old age on the activation of microglia/macrophage within the spinal cord after traumatic SCI remains incompletely understood. In a more recent experimental study, Hooshmand et al. compared aged female rats (18 months of age) to younger female rats (3 months of age) after moderate contusion SCI at T9 level using behavioral outcome measures and immunohistochemical analyses (Hooshmand et al., 2014). The authors concluded that aged rats had poorer locomotion recovery and increased proportion of injury area at 7 days to 28 days after SCI among aged rats when compared to younger rats (Hooshmand et al., 2014). However, there were no significant differences between the group of aged rats and the group of younger rats in terms of acute and chronic, humoral and cellular innate immune responses as assessed by serum complement activity, and neutrophil infiltration, respectively (Hooshmand et al., 2014). In fact, their reported results showed that aged rats did not significantly differ from younger rats with regards to the percentage of spared tissue and percentage area (i.e. the ratio of lesion area over cross sectional area of spinal cord) in the majority of the sections except for the section located 0.72 mm from the injury epicenter where aged animals had greater tissue injury area (Hooshmand et al., 2014).

Given this, our study provides important and original information on the inflammatory response to human SCI using a unique collection of postmortem spinal cord tissue, which confirms some of the findings from prior experimental studies. Generally speaking, the results of this study indicate that age at the time of injury does not influence cellular inflammatory response to traumatic SCI. This concept supports inclusion of elderly individuals with traumatic SCI in the clinical trials using neuroprotective strategies focused on modulation of neuroinflammation.

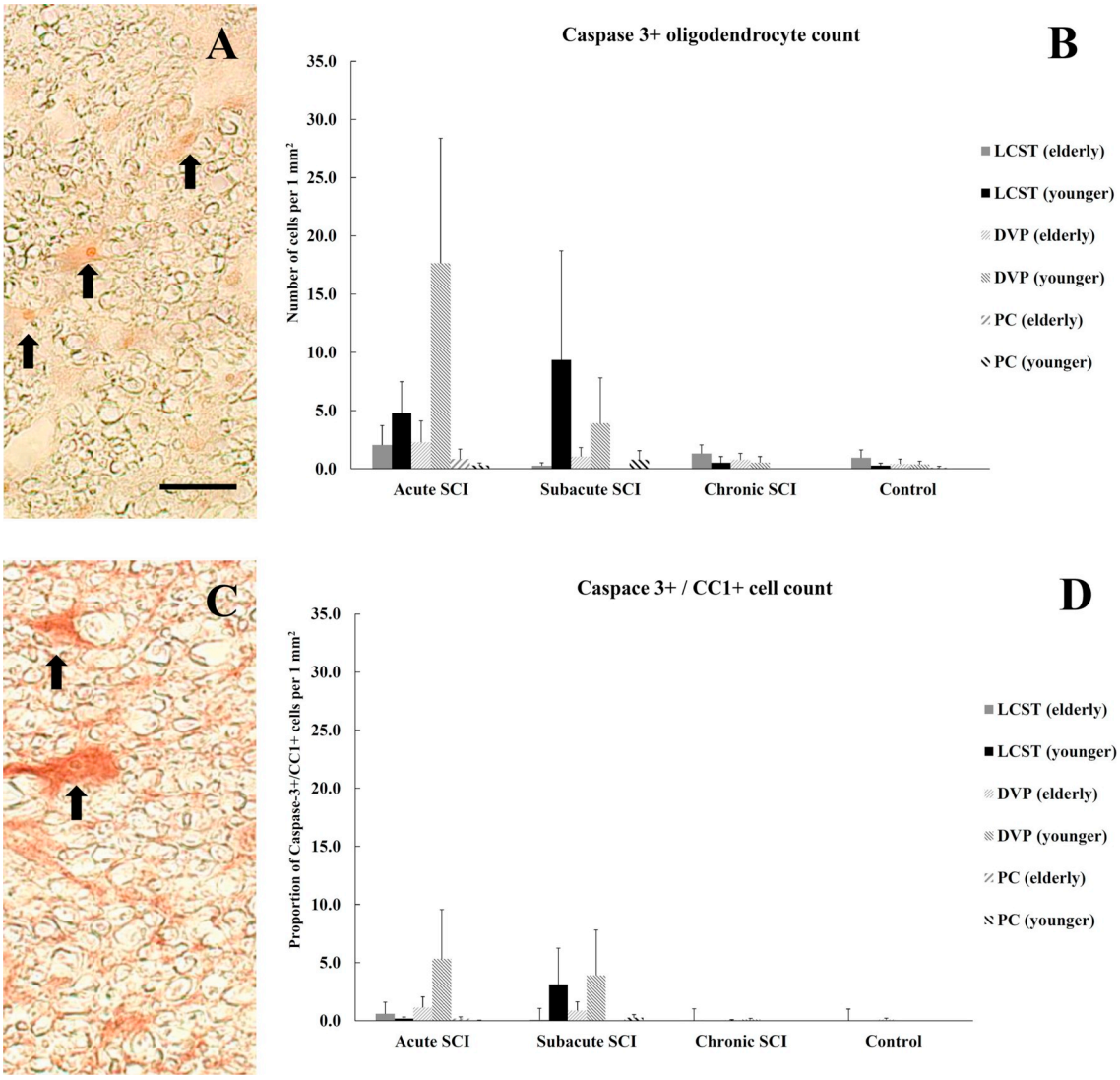


Fig. 3. Photomicrographs of representative areas of spinal cord white matter from a 67-year old male who suffered a severe C2 spinal cord injury (SCI) after fall and died 3.5 months post-trauma in high magnification (x40). The mid-cervical section was immunostained for apoptosis (Caspase 3+ cell with morphology of oligodendrocyte indicated by an arrow) (A), and oligodendrocyte (CC1+ cells indicated by arrows) (C). Calibration bar is 50 μm. Results of comparisons between younger and elderly individuals with regards to the number of apoptotic cells with morphology of oligodendrocytes (B) and proportion of Caspase 3+ cells over CC1+ cells (D) within the lateral corticospinal tracts (LCST), descending vasomotor pathways (DVP) and posterior column (PC) of spinal cord white matter in the acute, subacute and chronic stages following SCI as well as in control cases (all paired comparisons resulted in a $p > .05$). Data are represented as mean ± SEM.

4.2. Aging and oligodendroglial apoptosis after neurotrauma

While there are different theories on the role of apoptosis in the aging process, the exact mechanism of apoptosis in aging is not completely understood (Warner et al., 1997; Higami and Shimokawa, 2000). Prior experimental studies reported age-related differences on apoptosis in animal models, but no evidence in favor or against those findings was reported in humans to our knowledge (Lawson and Lowrie, 1998; Jiang et al., 2003). For instance, Zhao et al. reported "minimal" caspase-3 activation in aged individuals without history of CNS disease in comparison with significantly increased caspase-3 activation in postmortem brain tissue from individuals with Alzheimer's disease (Zhao et al., 2003). Numerous studies demonstrated apoptosis within the CNS after neurotrauma in animal models, but only a few investigations reported the occurrence of apoptosis in humans after traumatic brain injury and SCI (Emery et al., 1998; Coleman et al., 2000; Eldadah and Faden, 2000; Casha et al., 2001; Dumont et al., 2001; Keane et al., 2001; Hausmann et al., 2004).

The above mentioned animal study by Hooshmand et al. also compared aged female rats to younger female rats after moderate contusion SCI at T9 level with regards to the apoptotic cell death when examined cranially and caudally to the injury epicenter (Hooshmand et al., 2014). The authors concluded that aged rats had significantly greater number of cells in apoptosis, as identified by TUNEL immunostaining, than younger rats at 1.8 mm caudal to the injury epicenter, but their reported results also showed that there were no significant differences between the group of aged rats and the group of younger rats with regards to apoptotic cell death in all other caudal sections as well as at cranial sections, and injury epicenter sections (Hooshmand et al., 2014).

The results of the present study, therefore, deepen our understanding on the mechanisms underlying the potential age-related effects in spinal cord injured and uninjured individuals. The results of our study indicate that age at the time of injury did not significantly affect oligodendroglial apoptosis within the spinal cord white matter after traumatic SCI. In potentially translating neuroprotective therapeutic strategies involving inhibition of oligodendroglial apoptosis into human clinical trials, the results of this study provides key information on the

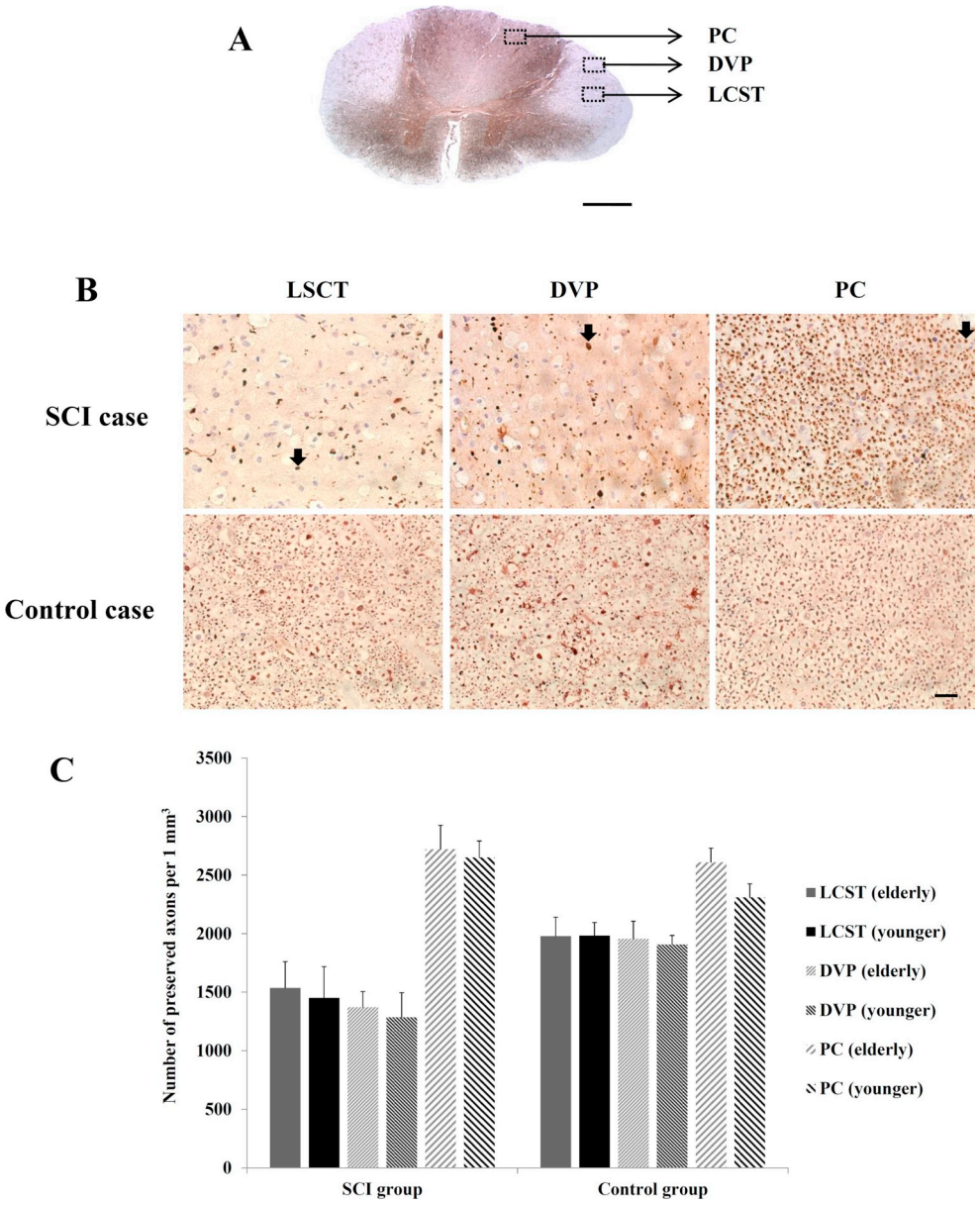


Fig. 4. Low-magnification (x1.25) photomicrograph of spinal cord from a 66-year old male who suffered a severe (motor and sensory complete) C6 spinal cord injury (SCI) and died 5 months post-trauma. A high thoracic spinal cord section was immunostained for axonal preservation with Neurofilament 200 and the studied areas are shown (calibration bar: 1 mm) (A). Representative areas of the lateral corticospinal tracts (LCST), descending vasomotor pathways (DVPs), and posterior column (PC) are shown in higher magnification (x40) with a few preserved axons indicated by arrows in the section that was taken two segments caudal to the level of SCI in a 45-year-old female who sustained a C6 severe SCI and died 1 year after her injury, or lower cervical spine segment in a control case, a 53-year-old female who died shortly after a ruptured brain aneurysm (calibration bar: 20 μm) (B). Comparisons between younger group and elderly group showed no significant differences ($p > .05$) with regards to the number of axons within the LCST, DVPs, and PC in the SCI group as well as in the control group (individuals without previous history of central nervous system (CNS) trauma) (C). Data are represented as mean ± SEM.

lack of significant effects of age at the SCI onset on oligodendroglial apoptosis.

4.3. Aging and axonal survival after neurotrauma

Axonal changes are well-recognized as a key predictor of neurological outcome in various human CNS conditions including SCI (Fehlings and Tator, 1995; Medana and Esiri, 2003). Progressive degenerative changes in the CNS and autonomic nervous system were associated with aging in certain animal models (Frolkis et al., 1997; Elder et al., 1999; Bergman and Ulfhake, 2002; Schmidt, 2002; Cowen et al., 2003). Prior histopathological studies suggested a number of aging effects on human spinal cord morphology (Tanaka, 1984; Kameyama et al., 1994). Using quantitative morphometric methods, Zhou et al. reported reduction of number of axons within the lateral corticospinal tracts in the lumbar segments of humans due to aging (Zhou et al., 1997). Terao et al. also demonstrated an indirect correlation between aging and the number of myelinated fibers within human corticospinal tracts in different spinal cord segments (Terao et al., 1994). Nonetheless, the results of a prior cases series using neuroanatomical analysis of postmortem spinal cord tissue ($n = 7$) revealed no significant age-related differences for extent of myelin degeneration or number of intact axons within sensory, motor and autonomic spinal cord tracts following acute motor complete cervical SCI (Furlan et al., 2010). Of note, the mean (± standard error of mean) time from injury to death was 9.2 ± 4.6 months (range from 5 weeks to 3 years) in that case series (Furlan et al., 2010).

Overall, the results of the present histopathological and immunocytochemical study analyzing a larger sample of postmortem human spinal cord tissue confirmed those previous results from the literature (Furlan et al., 2010).

4.4. Older age and outcomes after traumatic spinal cord injury

Using a large cohort of 485 individuals with acute traumatic SCI enrolled in the Second National Acute SCI Study (NASCIS-2 trial), the potential effects of old age on neurological recovery and mortality after SCI were previously studied (Furlan et al., 2010). The mortality rates in the elderly group were 10-fold higher than younger individuals in the acute and chronic stages after traumatic SCI. The reasons for a greater

mortality after SCI in the geriatric group include more frequent pre-existing medical comorbidities in the elderly when compared to younger individuals, even though other potential confounders may play a role such as ageistic attitudes and reduced physiological reserves (Furlan and Fehlings, 2009; Furlan et al., 2009a; Furlan et al., 2009b). Among survivors, age did not adversely affect motor and sensory recovery in the acute to chronic stages after SCI in unadjusted models and after controlling for potential major confounders (i.e. sex, ethnic group, Glasgow coma score, co-intervention, NASCIS-2 drug protocol, cause of injury, level and severity of SCI) (Furlan et al., 2010). Those results were similar to another study that included 499 individuals with acute traumatic SCI who were enrolled in the Third National Spinal Cord Injury Study (NASCIS 3 trial) (Furlan and Fehlings, 2009). While elderly individuals had the same potential to neurologically recover within the first year after acute traumatic SCI when compared to their younger counterparts, elderly individuals remained with greater degrees of disability after SCI (assessed using Functional Independence Measure) than younger individuals at 1 year following SCI (Furlan and Fehlings, 2009). In another retrospective cohort study, there were no significant age-related differences with regards to the degree of disability at the time of discharge from a tertiary rehabilitation center when the authors also considered the minimal clinically important differences for Spinal Cord Independence Measure and Functional Independence Measure in their data analyses (Furlan et al., 2013).

Altogether, the results of this neuroanatomical and immunohistochemical analysis of postmortem spinal cord tissue from 64 cases of SCI are consistent with those prior clinical studies that reported no significant effects of age at the SCI onset on the degree of impairment after traumatic SCI.

4.5. Study limitations

This clinically relevant study used a unique and relatively large sample of postmortem spinal cord tissue from individuals who died after traumatic SCI and from individuals without prior history of CNS injury. Although the analysis of postmortem spinal cord tissue provided key and original information on inflammatory response, oligodendroglial apoptosis and axonal survival after traumatic SCI, there are some methodological concerns that must be considered prior to the application of the study results. First, the method used to count cells and axons within white matter spinal cord tracts did not allow the stereological analysis of the sections with correction for volume and area (Long et al., 1999). Interestingly, the findings previous studies based on areal density that had suggested age-related cell number changes have been challenged by the results of more recent studies using new design-based stereological techniques (Long et al., 1999). Second, the cell counting within CNS tissue is particularly challenging in studies focused on aging because brain tissue from older individuals shrinks substantially less than tissue from younger individuals (Haug et al., 1984; Haug, 1986). Because similar effects putatively occur during fixation of spinal cord tissue, this differential shrinkage artifact actually favors our alternative hypothesis where cell and axon counting diminishes with aging (Furlan et al., 2010). Consequently, the potential effects of the tissue preparation artifact reinforce our negative results with regard to the influence of age on number of inflammatory cells, apoptotic oligodendrocytes, and preserved axons within the spinal cord white matter following traumatic SCI. Third, clinical data for all cases included in this case series were limited to age, sex, and previous medical history that are available in the Toronto Western Hospital Spinal Cord Tissue Bank and Miami Project Spinal Cord Tissue Bank. Therefore, our data analyses were not controlled for some other potential confounding effects such as the use of drugs (especially, the use of corticosteroids), that could affect neuroinflammatory response and/or mitigate secondary injury response after SCI. Fourth, sections of the postmortem spinal cord tissues obtained for this study could not reliably be double immunostained with CC1, as a marker for oligodendrocytes, and Caspase-3, as a marker for apoptosis. As an alternative, we counted apoptotic cells (Caspace-3+ cells) with morphology of oligodendrocytes as well as the number of oligodendrocytes (CC1+ cells) within selected areas of the spinal cord white matter. Fifth, data on the severity of SCI were missing in a large number of the cases in our cohort, which precluded including this important potential confounder in the regression models. Using data from 2019 Annual Statistical Report published by the National Spinal Cord Injury Statistical Center, the severity of SCI was found to be correlated with the cause of injury (data not shown) and, hence, the latter was used as an approximate surrogate for the former in our regression models (NSCISC, 2019). Finally, this study was focused on the counting of the number of inflammatory cells within the spinal cord white matter, however it remains unclear whether age at the time of SCI affects the activity of those inflammatory cells because their function was not assessed in this study. This is also important matter that deserves further investigations since, for instance, some T cells regulate the release of anti-inflammatory cytokines, which prevent excessive CNS inflammation (Di Benedetto et al., 2017). Also, increased levels of interleukin 6 (IL-6) promotes differentiation of macrophages into their inflammatory M1-phenotype, whereas elevation of the IL-4 levels prompts differentiation of macrophages into their anti-inflammatory M2-phenotype (Di Benedetto et al., 2017).

5. Conclusions

The results of this histopathological and immunohistochemical study indicate that age at the SCI onset does not adversely affect the cellular inflammatory response to, oligodendroglial apoptosis and axonal survival after traumatic SCI. Those results are consistent with prior clinical studies that have shown no significant effects of age on neurological and functional recovery following traumatic SCI when data analyses are adjusted for potential confounders. This study also provides important information on the inflammatory response to human SCI using a unique collection of postmortem spinal cord tissue. By determining the effects of age on neuroinflammation and axonal changes in spinal cord injured and uninjured individuals, this study can have an impact on the current protocols for rehabilitation, cell-based therapies and neuroprotective strategies to maximize recovery following acute traumatic SCI (Jacobs and Fehlings, 2003; Gris et al., 2004; Saville et al., 2004; Austin and Fehlings, 2008; Baptiste and Fehlings, 2008). Indeed, the results of this study support the notion that elderly individuals can potentially have similar benefits to younger individuals of the ongoing translational studies focused on neuroprotective strategies based on modulation of neuroinflammation or inhibition of oligodendroglial apoptosis.

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Author contributions

Dr. Julio Furlan was responsible for conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; data interpretation; project administration; and writing original draft and revising the manuscript. Dr. Yang Liu was responsible for conceptualization; data curation; formal analysis; methodology; data interpretation; project administration; and editing the manuscript. Dr. W. Dalton Dietrich III was responsible for conceptualization; data curation; methodology; data interpretation; project administration; and editing

the manuscript. Dr. Michael D. Norenberg was responsible for conceptualization; data curation; methodology; data interpretation; project administration; and editing the manuscript. Dr. Michael Fehlings was responsible for conceptualization; data curation; methodology; data interpretation; supervision; project administration; and editing the manuscript.

Declaration of Competing Interest

The authors declare no competing interests to disclose.

References

Allison, D.J., Ditor, D.S., 2015. Immune dysfunction and chronic inflammation following spinal cord injury. Spinal Cord 53, 14–18.

Austin, J.W., Fehlings, M.G., 2008. Molecular mechanisms of Fas-mediated cell death in oligodendrocytes. J. Neurotrauma 25, 411–426.

Baptiste, D.C., Fehlings, M.G., 2008. Emerging drugs for spinal cord injury. Expert Opin. Emerg. Drugs 13, 63–80.

Bergman, E., Ulfhake, B., 2002. Evidence for loss of myelinated input to the spinal cord in senescent rats. Neurobiol. Aging 23, 271–286.

Caffo, M., Caruso, G., Germano, A., Galatioto, S., Meli, F., Tomasello, F., 2005. CD68 and CR3/43 immunohistochemical expression in secretory meningiomas. Neurosurgery 57, 551–557 (discussion 551–557).

Casha, S., Yu, W.R., Fehlings, M.G., 2001. Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression following spinal cord injury in the rat. Neuroscience 103, 203–218.

Catz, A., Thaleisnik, M., Fishel, B., Ronen, J., Spasser, R., Fredman, B., Shabtay, E., Gepstein, R., 2002. Survival following spinal cord injury in Israel. Spinal Cord 40, 595–598.

Charriaut-Marlangue, C., Ben-Ari, Y., 1995. A cautionary note on the use of the TUNEL stain to determine apoptosis. Neuroreport 7, 61–64.

Coleman, W.P., Benzel, D., Cahill, D.W., Ducker, T., Geisler, F., Green, B., Gropper, M.R., Goffin, J., Madsen III, P.W., Maiman, D.J., Ondra, S.L., Rosner, M., Sasso, R.C., Trost, G.R., Zeidman, S., 2000. A critical appraisal of the reporting of the National Acute Spinal Cord Injury Studies (II and III) of methylprednisolone in acute spinal cord injury. J. Spinal Disord. 13, 185–199.

Conde, J.R., Streit, W.J., 2006. Microglia in the aging brain. J. Neuropathol. Exp. Neurol. 65, 199–203.

Cowen, T., Woodhoo, A., Sullivan, C.D., Jolly, R., Crutcher, K.A., Wyatt, S., Michael, G.J., Orike, N., Gatzinsky, K., Thrasivoulou, C., 2003. Reduced age-related plasticity of neurotrophin receptor expression in selected sympathetic neurons of the rat. Aging Cell 2, 59–69.

Czesnikiewicz-Guzik, M., Lee, W.W., Cui, D., Hiruma, Y., Lamar, D.L., Yang, Z.Z., Ouslander, J.G., Weyand, C.M., Goronzy, J.J., 2008. T cell subset-specific susceptibility to aging. Clin. Immunol. 127, 107–118.

Deininger, M.H., Pater, S., Strik, H., Meyermann, R., 2001. Macrophage/microglial cell subpopulations in glioblastoma multiforme relapses are differentially altered by radiochemotherapy. J. Neuro-Oncol. 55, 141–147.

DeVivo, M.J., Krause, J.S., Lammertse, D.P., 1999. Recent trends in mortality and causes of death among persons with spinal cord injury. Arch. Phys. Med. Rehabil. 80, 1411–1419.

Di Benedetto, S., Muller, L., Wenger, E., Duzel, S., Pawelec, G., 2017. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. Neurosci. Biobehav. Rev. 75, 114–128.

Dumont, R.J., Okonkwo, D.O., Verma, S., Hurlbert, R.J., Boulos, P.T., Ellegala, D.B., Dumont, A.S., 2001. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin. Neuropharmacol. 24, 254–264.

Eldadah, B.A., Faden, A.I., 2000. Caspase pathways, neuronal apoptosis, and CNS injury. J. Neurotrauma 17, 811–829.

Elder, G.A., Friedrich Jr., V.L., Margita, A., Lazzarini, R.A., 1999. Age-related atrophy of motor axons in mice deficient in the mid-sized neurofilament subunit. J. Cell Biol. 146, 181–192.

Emery, E., Aldana, P., Bunge, M.B., Puckett, W., Srinivasan, A., Keane, R.W., Bethea, J., Levi, A.D., 1998. Apoptosis after traumatic human spinal cord injury. J. Neurosurg. 89, 911–920.

Fehlings, M.G., Tator, C.H., 1995. The relationships among the severity of spinal cord injury, residual neurological function, axon counts, and counts of retrogradely labeled neurons after experimental spinal cord injury. Exp. Neurol. 132, 220–228.

Fleming, J.C., Norenberg, M.D., Ramsay, D.A., Dekaban, G.A., Marcillo, A.E., Saenz, A.D., Pasquale-Styles, M., Dietrich, W.D., Weaver, L.C., 2006. The cellular inflammatory response in human spinal cords after injury. Brain 129, 3249–3269.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C., Salvioli, S., 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev. 128, 92–105.

Frolkis, V.V., Tanin, S.A., Gorban, Y.N., 1997. Age-related changes in axonal transport. Exp. Gerontol. 32, 441–450.

Furlan, J.C., Fehlings, M.G., 2009. The impact of age on mortality, impairment, and disability among adults with acute traumatic spinal cord injury. J. Neurotrauma 26, 1707–1717.

Furlan, J.C., Tator, C.H., 2012. Global epidemiology of traumatic spinal cord injury. In: Morganti-Kossman, C., Raghupathi, R., Maas, A. (Eds.), Traumatic Brain and Spinal Cord Injury: Challenges and Developments. Cambridge University Press, Cambridge, First edition, pp. 360.

Furlan, J.C., Fehlings, M.G., Shannon, P., Norenberg, M.D., Krassioukov, A.V., 2003. Descending vasomotor pathways in humans: correlation between axonal preservation and cardiovascular dysfunction after spinal cord injury. J. Neurotrauma 20, 1351–1363.

Furlan, J.C., Krassioukov, A.V., Fehlings, M.G., 2006. Hematologic abnormalities within the first week after acute isolated traumatic cervical spinal cord injury: a case-control cohort study. Spine (Phila Pa 1976) 31, 2674–2683.

Furlan, J.C., Craven, B.C., Ritchie, R., Coukos, L., Fehlings, M.G., 2009a. Attitudes towards the older patients with spinal cord injury among registered nurses: a cross-sectional observational study. Spinal Cord 47, 674–680.

Furlan, J.C., Kattail, D., Fehlings, M.G., 2009b. The impact of co-morbidities on age-related differences in mortality after acute traumatic spinal cord injury. J. Neurotrauma 26, 1361–1367.

Furlan, J.C., Bracken, M.B., Fehlings, M.G., 2010. Is age a key determinant of mortality and neurological outcome after acute traumatic spinal cord injury? Neurobiol. Aging 31, 434–446.

Furlan, J.C., Hitzig, S.L., Craven, B.C., 2013. The influence of age on functional recovery of adults with spinal cord injury or disease after inpatient rehabilitative care: a pilot study. Aging Clin. Exp. Res. 25, 463–471.

Gris, D., Marsh, D.R., Oatway, M.A., Chen, Y., Hamilton, E.F., Dekaban, G.A., Weaver, L.C., 2004. Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. J. Neurosci. 24, 4043–4051.

Gris, D., Hamilton, E.F., Weaver, L.C., 2008. The systemic inflammatory response after spinal cord injury damages lungs and kidneys. Exp. Neurol. 211, 259–270.

Grubeck-Loebenstein, B., Wick, G., 2002. The aging of the immune system. Adv. Immunol. 80, 243–284.

Haug, H., 1986. History of neuromorphometry. J. Neurosci. Methods 18, 1–17.

Haug, H., Kuhl, S., Mecke, E., Sass, N.L., Wasner, K., 1984. The significance of morphometric procedures in the investigation of age changes in cytoarchitectonic structures of human brain. J. Hirnforsch. 25, 353–374.

Hausmann, R., Biermann, T., Wiest, I., Tubel, J., Betz, P., 2004. Neuronal apoptosis following human brain injury. Int. J. Legal Med. 118, 32–36.

Hayes, K.C., Kakulas, B.A., 1997. Neuropathology of human spinal cord injury sustained in sports-related activities. J. Neurotrauma 14, 235–248.

Higami, Y., Shimokawa, I., 2000. Apoptosis in the aging process. Cell Tissue Res. 301, 125–132.

Hooshmand, M.J., Galvan, M.D., Partida, E., Anderson, A.J., 2014. Characterization of recovery, repair, and inflammatory processes following contusion spinal cord injury in old female rats: is age a limitation? Immun. Ageing 11, 15.

Hughes, S., Gardiner, T., Hu, P., Baxter, L., Rosinova, E., Chan-Ling, T., 2006. Altered pericyte-endothelial relations in the rat retina during aging: implications for vessel stability. Neurobiol. Aging 27, 1838–1847.

Iancu, E.M., Speiser, D.E., Rufer, N., 2008. Assessing ageing of individual T lymphocytes: mission impossible? Mech. Ageing Dev. 129, 67–78.

Jacobs, W.B., Fehlings, M.G., 2003. The molecular basis of neural regeneration. Neurosurgery 53, 943–948 (discussion 948–950).

Jiang, X., Edstrom, E., Altun, M., Ulfhake, B., 2003. Differential regulation of Shc adaptor proteins in skeletal muscle, spinal cord and forebrain of aged rats with sensorimotor impairment. Aging Cell 2, 47–57.

Kahveci, Z., Minbay, F.Z., Noyan, S., Cavusoglu, I., 2003. A comparison of microwave heating and proteolytic pretreatment antigen retrieval techniques in formalin fixed, paraffin embedded tissues. Biotech. Histochem 78, 119–128.

Kameyama, T., Hashizume, Y., Ando, T., Takahashi, A., 1994. Morphometry of the normal cadaveric cervical spinal cord. Spine (Phila Pa 1976) 19, 2077–2081.

Keane, R.W., Kraydieh, S., Lotocki, G., Alonso, O.F., Aldana, P., Dietrich, W.D., 2001. Apoptotic and antiapoptotic mechanisms after traumatic brain injury. J. Cereb. Blood Flow Metab. 21, 1189–1198.

Krassioukov, A.V., Weaver, L.C., 1996. Morphological changes in sympathetic preganglionic neurons after spinal cord injury in rats. Neuroscience 70, 211–225.

Lawson, S.J., Lowrie, M.B., 1998. The role of apoptosis and excitotoxicity in the death of spinal motoneurons and interneurons after neonatal nerve injury. Neuroscience 87, 337–348.

Lemstra, A.W., Groen in't Woud, J.C., Hoozemans, J.J., van Haastert, E.S., Rozemuller, A.J., Eikelenboom, P., van Gool, W.A., 2007. Microglia activation in sepsis: a case-control study. J. Neuroinflammation 4, 4.

Long, J.M., Mouton, P.R., Jucker, M., Ingram, D.K., 1999. What counts in brain aging? Design-based stereological analysis of cell number. J. Gerontol. A Biol. Sci. Med. Sci. 54, B407–B417.

Medana, I.M., Esiri, M.M., 2003. Axonal damage: a key predictor of outcome in human CNS diseases. Brain 126, 515–530.

Moody, D.M., Santamore, W.P., Bell, M.A., 1991. Does tortuosity in cerebral arterioles impair down-autoregulation in hypertensives and elderly normotensives? A hypothesis and computer model. Clin. Neurosurg. 37, 372–387.

Mun-Bryce, S., Rosenberg, G.A., 1998. Gelatinase B modulates selective opening of the blood-brain barrier during inflammation. Am. J. Phys. 274, R1203–R1211.

Nashmi, R., Fehlings, M.G., 2001. Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord. Neuroscience 104, 235–251.

Noble, L.J., Donovan, F., Igarashi, T., Goussev, S., Werb, Z., 2002. Matrix

metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. J. Neurosci. 22, 7526–7535.
NSCISC, 2004. The 2004 Annual Statistical Report for the mModel Spinal Cord Injury Care Systems. National Spinal Cord Injury Statistical Center, Birmingham, pp. 1–176.
NSCISC, 2019. Annual Statistical Report for the Spinal Cord Injury Model Systems Public Version. National Spinal Cord Injury Statistical Center, Birmingham, Alabama, pp. 1–126.
O'Connor, P.J., 2005. Survival after spinal cord injury in Australia. Arch. Phys. Med. Rehabil. 86, 37–47.
Puckett, W.R., Hiester, E.D., Norenberg, M.D., Marcillo, A.E., Bunge, R.P., 1997. The astroglial response to Wallerian degeneration after spinal cord injury in humans. Exp. Neurol. 148, 424–432.
Qiu, M.G., Zhu, X.H., 2004. Aging changes of the angioarchitecture and arterial morphology of the spinal cord in rats. Gerontology 50, 360–365.
Ramprasad, M.P., Terpstra, V., Kondratenko, N., Quehenberger, O., Steinberg, D., 1996. Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. Proc. Natl. Acad. Sci. U. S. A. 93, 14833–14838.
Saville, L.R., Pospisil, C.H., Mawhinney, L.A., Bao, F., Simedrea, F.C., Peters, A.A., O'Connell, P.J., Weaver, L.C., Dekaban, G.A., 2004. A monoclonal antibody to CD11d reduces the inflammatory infiltrate into the injured spinal cord: a potential neuroprotective treatment. J. Neuroimmunol. 156, 42–57.
Schmidt, R.E., 2002. Age-related sympathetic ganglionic neuropathology: human pathology and animal models. Auton. Neurosci. 96, 63–72.
Shi, S.R., Cote, R.J., Taylor, C.R., 2001. Antigen retrieval techniques: current perspectives. J. Histochem. Cytochem. 49, 931–937.
Sloane, J.A., Hollander, W., Moss, M.B., Rosene, D.L., Abraham, C.R., 1999. Increased microglial activation and protein nitration in white matter of the aging monkey. Neurobiol. Aging 20, 395–405.
Staines, W.A., Meister, B., Melander, T., Nagy, J.I., Hokfelt, T., 1988. Three-color immunofluorescence histochemistry allowing triple labeling within a single section. J. Histochem. Cytochem. 36, 145–151.
Streit, W.J., 2005. Microglia and neuroprotection: implications for Alzheimer's disease. Brain Res. Brain Res. Rev. 48, 234–239.
Streit, W.J., Conde, J.R., Fendrick, S.E., Flanary, B.E., Mariani, C.L., 2005. Role of microglia in the central nervous system's immune response. Neurol. Res. 27, 685–691.
Tanaka, Y., 1984. Morphological changes of the cervical spinal canal and cord due to aging. Nihon Seikeigeka Gakkai Zasshi 58, 873–886.
Tandrup, T., 2004. Unbiased estimates of number and size of rat dorsal root ganglion cells in studies of structure and cell survival. J. Neurocytol. 33, 173–192.
Terao, S., Sobue, G., Hashizume, Y., Shimada, N., Mitsuma, T., 1994. Age-related changes of the myelinated fibers in the human corticospinal tract: a quantitative analysis. Acta Neuropathol. 88, 137–142.
Warner, H.R., Hodes, R.J., Pocinki, K., 1997. What does cell death have to do with aging? J. Am. Geriatr. Soc. 45, 1140–1146.
Weng, N.P., 2006. Aging of the immune system: how much can the adaptive immune system adapt? Immunity 24, 495–499.
Yung, R.L., Julius, A., 2008. Epigenetics, aging, and autoimmunity. Autoimmunity 41, 329–335.
Zhao, M., Su, J., Head, E., Cotman, C.W., 2003. Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease. Neurobiol. Dis. 14, 391–403.
Zhou, M., Goto, N., Otsuka, N., Moriyama, H., Nakamura, Y., 1997. Morphometric analyses of axons in the lateral corticospinal tract with ageing process. Okajimas Folia Anat. Jpn. 74, 133–138.