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 SCI, 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 at the onset 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 (Krausiov 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, oligodendrogial 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 postmortem 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 mononuclear 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, Novocasta 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 ABC (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 NovoRED 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 vasmotor 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 vasmotor 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 \((p = .049; \text{Fig. 1B})\). 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 \((p = .016; \text{Fig. 1D})\). Furthermore, there was a trend towards a higher density of infiltrated B-cell lymphocytes within the lateral corticospinal tracts among the younger individuals when compared with elderly individuals in the subacute stage following SCI \((p = .059; \text{Fig. 2B})\). 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 \((p = .049; \text{Fig. 2D})\).

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.

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

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 immunostaining 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.
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 hematogenous 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 in humans after SCI and, reportedly, reached a peak between 1 and 7 days post-injury (Fleming et al., 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 a more delayed 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.
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
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 based on modulation of neuroinflammation or inhibition of oligodendroglial apoptosis. 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.

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