the incidence of infections. In contrast, G-CSF increases the number of white blood cells in the peripheral blood. This feature of G-CSF is used clinically to treat neutropenia and prevent infectious complications. In this manner, G-CSF treatment might decrease the incidence of infections in SCI patients. Previous studies have reported a 31.4 % incidence of pneumonia in SCI patients with a Frankel Grade of A, B, or C [15]. Matsumoto reported a 30.4 % incidence of pneumonia and a 4.3 % incidence of urinary tract infections in SCI patients who received MPSS [16]. We cannot directly compare the incidence of infections between the present study and previous reports, and the anti-infection properties of G-CSF remain to be clarified, but our findings suggest that the incidence of pneumonia might be reduced in patients treated with G-CSF compared to those treated with MPSS.

We observed a lower incidence of gastric ulcers in patients treated with G-CSF than in patients treated MPSS. When we analyzed the incidence of ulcers among patients with severe incomplete paralysis (AIS grades B and C) to exclude the bias introduced by the difference in paralysis severity between the groups, no significant difference was observed. Treatment of gastric ulcers has been dramatically improved by the increased use of proton pump inhibitors. Our results might thus reflect this change in ulcer prophylaxis and treatment.

Those findings suggest that G-CSF treatment has a lower risk of severe adverse events than MPSS treatment. Hence, G-CSF may be a reasonable alternative to MPSS, but a direct comparison of the efficacy of each drug is needed.

As for the cost, the price of G-CSF (300 μg) in Japan is 24,926 yen (175.2 Euro in the rate of Jan. 26, 2014).We employed the G-CSF dose regimen of 10 μg/kg/d × 5 days. Therefore, the total cost of G-CSF therapy in patient with 60 kg body weight is 249,260 yen (1,752.05 Euro). MPSS (500 mg) costs 3,536 yen (24.85 Euro). The dose regimen of MPSS in NASCIS 2 is 5.4 mg/kg as a bolus injection followed by 5.4 mg/kg/h for 23 h. Therefore, the total cost of MPSS therapy protocol in patient with 60 kg body weight is 67,184 yen (472.17 Euro). The cost of G-CSF therapy is higher than that in the MPSS therapy, of which difference in total cost is 182,816 yen (1,284.83 Euro).

The present study has several major limitations. First, the patients were not randomly allocated to the treatment groups. Second, the control group was historical. Third, the number of patients was too small to prove the efficacy of G-CSF treatment with sufficient statistical power. Finally, the timing of treatment initiation differed between treatment groups (within 8 h after injury in the MPSS group and within 48 h after injury in the G-CSF group).

The results of the current study suggest that G-CSF administration is both safe and effective. Although we cannot draw conclusions about the efficacy of G-CSF without prospective randomized controlled trial, the present results encourage us to make step forward to perform next phase of clinical trial.

Conflict of interest None.

References

1. Bauchet L, Lonjon N, Perrin FE et al (2009) Strategies for spinal cord repair after injury: a review of the literature and information. Ann Phys Rehabil Med 52:330–351
2. Park E, Velumian A, Fehlings MG (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21:754–774
3. Varma AK, Das A, Wallace G 4th et al (2013) Spinal Cord Injury: a review of current therapy, future treatments, and basic science frontiers. Neurochem Res 38:895–905
4. Bracken MB, Shepard MJ, Collins WF et al (1990) A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury: results of the second national acute spinal cord injury study. N Engl J Med 322:1405–1411
5. Bracken MB (2012) Steroids for acute spinal cord injury. Cochrane Database Syst Rev. doi:10.1002/14651858
6. Hurlbert RJ, Hadley MN, Walters BC et al (2013) Pharmacological therapy for acute spinal cord injury. Neurosurgery 72(Suppl 2):93–105. doi:10.1227/NEU.0b013e31827765c6
7. Nicola NA, Metcalf D, Matsumoto M et al (1983) Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. J Biol Chem 258:9017–9023
8. Roberts AW (2005) G-CSF: a key regulator of neutrophil production, but that's no all! Growth Factors 23:33–41
9. Kawabe J, Koda M, Hashimoto M et al (2011) Neuroprotective effects of granulocyte colony-stimulating factor and relationship to promotion of angiogenesis after spinal cord injury in rats. J Neurosurg Spine 15:414–421. doi:10.3171/2011.5.SPINE10421
10. Koda M, Nishio Y, Kamada T et al (2007) Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice. Brain Res 1149:223–231
11. Nishio Y, Koda M, Kamada T et al (2007) Granulocyte colony-stimulating factor (G-CSF) attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. J Neuropathol Exp Neurol 66:724–731
12. Kadota R, Koda M, Kawabe J et al (2012) Granulocyte Colony-Stimulating Factor (G-CSF) Protects Oligpdendrocyte and promotes hindlimb functional recovery after spinal cord injury in rats. PLoS One 7:e50391. doi:10.1371/journal.pone.0050391
13. Takahashi H, Yamazaki M, Okawa A et al (2012) Neuroprotective therapy using granulocyte colony-stimulating factor for acute spinal cord injury: a phase I/IIa clinical trial. Eur Spine J 21:2580–2587. doi:10.1007/s00586-012-2213-3
14. Kliesch WF, Cruse JM, Lewis RE et al (1996) Restoration of depressed immune function in spinal cord injury patients receiving rehabilitation therapy. Paraplegia 34:82–90
15. Jackson AB, Groomes TE (1994) Incidence of respiratory complications following spinal cord injury. Arch Phys Med Rehabil 75:270–275
16. Matsumoto T, Tamaki T, Kawakami M et al (2001) Early complications of high-dose methyl-prednisolone sodium succinate treatment in the follow-up of acute cervical spinal cord injury. Spine 26:426–430

Chapter 13
Granulocyte Colony-Stimulating Factor-Mediated Neuroprotective Therapy for Spinal Cord Injury

Masao Koda, Takeo Furuya, Taigo Ianada, Koshiro Kamiya, Mitsutoshi Ota, Satoshi Maki, Akihiko Okawa, Kazuhisa Takahashi, and Masashi Yamazaki

Abstract To prove the efficacy of granulocyte colony-stimulating factor (G-CSF) for spinal cord injury (SCI), we performed several animal experiments in rodent SCI models. Through those experiments, we showed G-CSF's mechanisms of action for SCI.

G-CSF showed efficacy for SCI through mobilization of bone marrow-derived cells. G-CSF attenuated neuronal cell death in vitro and in vivo, resulting in promotion of functional recovery after SCI. Expression of IL-1β and TNF-α was significantly suppressed by G-CSF in the acute phase of SCI. G-CSF promoted upregulation of anti-apoptotic protein Bcl-Xl on oligodendrocytes and suppressed apoptosis of oligodendrocytes after SCI. G-CSF exerted neuroprotective effects via promotion of angiogenesis after SCI.

G-CSF's current use in the clinic for hematopoietic stimulation and its ongoing clinical trial for brain infarction make it an appealing molecule that could be rapidly placed into trials for acute SCI patients. G-CSF is one of the hopeful candidates for clinical application.

Keywords G-CSF • Neuroprotection • Secondary injury

13.1 Introduction

The pathologies following acute spinal cord injury (SCI) are divided into two sequential events: the primary injury and the secondary injury [1]. Direct mechanical trauma induces the primary injury, which includes the spinal cord tissue damage.

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This initial insult then triggers a progressive wave of secondary injury, which exacerbates the injury to the spinal cord via the activation of pathophysiological mechanisms.

Known pathophysiological mechanisms of the secondary injury after SCI include ischemia, posttraumatic inflammatory response mediated by resident microglia and blood-derived inflammatory cells, release of excitatory amino acids, generation of reactive oxygen species, influx of Ca^{2+}, and so on [1]. Those multiple mechanisms instigate neuronal and glial cell death, resulting in exaggeration of tissue damage after SCI.

Secondary injury is the main therapeutic target for various kinds of drug therapies. Thus a huge effort has been expended by clinicians, basic scientists, and industry to discover effective neuroprotective agents which can act against mechanisms of the secondary injury following SCI [1].

Currently, high-dose methylprednisolone sodium succinate (MPSS) is the only clinically available treatment for acute SCI to reduce the secondary injury. In recent years, however, the use of high-dose MPSS in acute SCI has become controversial, largely based on the risk of serious adverse effects versus what is perceived to be a modest neurological benefit [2]. Therefore, development of new SCI drug therapies that could replace high-dose MPSS is an area of intense study.

Granulocyte colony-stimulating factor (G-CSF) is a 19.6 kDa glycoprotein that was identified initially as a serum component that induced differentiation of the murine myelomonocytic leukemic cell line and is capable of inducing the survival, proliferation, and differentiation of cells of neutrophil lineage [3, 4]. In addition to its effects as a hematopoietic cytokine, it was recently reported that G-CSF has the potential to promote the survival of other types of cells, including in ischemic myocardium [5]. In the central nervous system, G-CSF has direct neuroprotective effects against glutamate-induced neuronal death and stroke [6, 7]. Most recently, clinical trials have reported on the safety and feasibility of G-CSF administration following stroke, supporting the hypothesis that G-CSF may also be an effective therapeutic for SCI [8].

To prove the efficacy of G-CSF for SCI, we performed several animal experiments in rodent SCI models. Here we show the results of those experiments, indicating G-CSF's mechanism of action for SCI.

13.1.1 G-CSF Receptor Expression

To assess the expression of G-CSF receptor (G-CSFR), we performed immunofluorescence double staining on histological sections of spinal cords. The data revealed that G-CSFR was expressed on neurons, astrocytes, and oligodendrocytes in normal spinal cord (Fig. 13.1). According to the expression pattern of G-CSFR, we speculated that G-CSF can act on neuron, astrocyte, and oligodendrocyte.

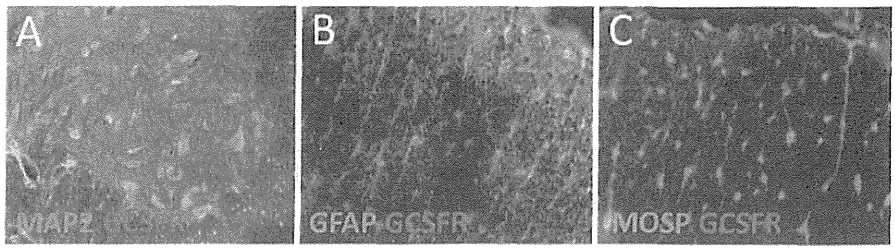

Fig. 13.1 Expression of granulocyte colony-stimulating factor receptor (G-CSFR) in normal spinal cord. Immunohistochemistry for G-CSFR and cell type markers was performed. G-CSFR was expressed by MAP2+ neurons (a), GFAP+ astrocytes (b), and MOSP+ oligodendrocytes (c)

13.1.2 G-CSF Promotes Migration of Bone Marrow-Derived Stem Cells into Injured Spinal Cord

To elucidate the effects of G-CSF-mediated mobilization of bone marrow-derived stem cells on the injured spinal cord, we constructed bone marrow chimera mice. Bone marrow cells of green fluorescent protein (GFP) transgenic mice were transplanted into lethally irradiated C57BL/6 mice. Four weeks after bone marrow transplantation, a large part of the bone marrow cells of those chimera mice was GFP-positive, enabling the tracking of bone marrow-derived cells by green fluorescence. SCI was produced by a static load (20 g, 5 min) at T8 level on those chimera mice. G-CSF (200 μg/kg/d) was injected subcutaneously for 5 days. Immunohistochemistry for GFP and cell lineage markers was performed to evaluate G-CSF-mediated mobilization of bone marrow-derived cells into injured spinal cord. Hind limb locomotor recovery was assessed for 6 weeks.

Immunohistochemistry revealed that G-CSF increased the number of GFP-positive cells in injured spinal cord, indicating that G-CSF promoted mobilization of bone marrow-derived cells and enhanced migration of those cells into injured spinal cord. The numbers of double-positive cells for GFP and glial markers were larger in the G-CSF-treated mice than in the control mice. G-CSF-treated mice showed significant recovery of hind limb function compared to that of the control mice. G-CSF showed efficacy for SCI treatment through mobilization of bone marrow-derived cells [9].

13.1.3 G-CSF Suppresses Apoptosis of Neurons After SCI

To elucidate the direct neuroprotective effect of G-CSF, we performed in vitro experiments using cultured neurons and in vivo experiments using mouse compressive SCI model. We found that G-CSF is neuroprotective against glutamate-induced cell death of cerebellar granule neurons in vitro.

Next, we used a mouse model of compressive SCI to examine the neuroprotective potential of G-CSF in vivo. Histological assessment with cresyl violet staining

revealed that the number of surviving neurons in the injured spinal cord was significantly increased in G-CSF-treated mice. Immunohistochemistry for neuronal apoptosis revealed that G-CSF suppressed neuronal apoptosis after SCI. Moreover, administration of G-CSF promoted hind limb functional recovery. G-CSF might promote functional recovery by inhibiting neuronal apoptosis after SCI [10].

13.1.4 G-CSF Suppresses Inflammatory Cytokine Expression After SCI

To elucidate the potential therapeutic effect of G-CSF for SCI in rats, rat contusive SCI was introduced using the infinite horizon impactor (magnitude, 200 kilodyne). Recombinant human G-CSF (15.0 μg/kg) was administered by tail vein injection for 5 days. To detect the anti-inflammatory effects of G-CSF in the SCI model, we performed real-time PCR for inflammatory cytokines on the spinal cord sample of G-CSF and control rats. Twelve hours after surgery, expression of IL-1β and TNF-α mRNAs was significantly suppressed in the G-CSF group compared to the vehicle control group. The results of real-time PCR for the other factors showed no significant difference between the vehicle and G-CSF-treated groups. According to these results, G-CSF suppresses inflammatory cytokine expression after SCI [11].

13.1.5 G-CSF Suppresses Apoptosis of Oligodendrocytes and Protects Myelin After SCI

To elucidate anti-apoptotic effect of G-CSF on oligodendrocyte, in vivo experiments using rat contusive SCI introduced by the IH impactor (200 kilodyne) were performed. Recombinant human G-CSF (15.0 μg/kg) was administered by tail vein injection for 5 days. Histological assessment with luxol fast blue staining revealed that the area of white matter spared in the injured spinal cord was significantly larger in G-CSF-treated rats. Immunohistochemical analysis showed that G-CSF promoted upregulation of anti-apoptotic protein Bcl-Xl on oligodendrocytes and suppressed apoptosis of oligodendrocytes after SCI (Fig. 13.2). Moreover, administration of G-CSF promoted better functional recovery of hind limbs assessed by BBB locomotor scale [11].

13.1.6 G-CSF Promotes Angiogenesis After SCI

Because the degree of angiogenesis in the subacute phase after SCI correlates with regenerative responses, it is possible that G-CSF's neuroprotective effects after SCI are due to enhancement of angiogenesis. We utilized the contusive SCI rat model using IH impactor and randomly divided subjects between a G-CSF-treated group and a control group. In the G-CSF-treated rats, the total number of vessels was

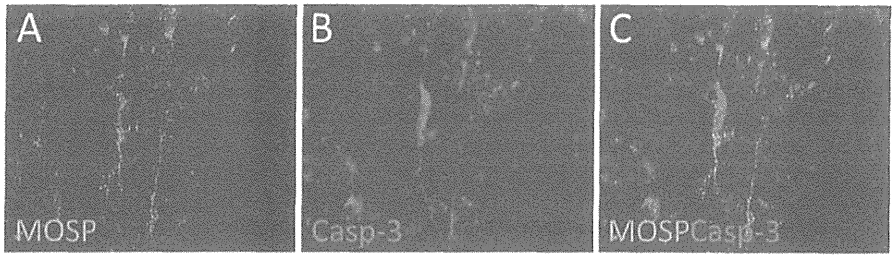

Fig. 13.2 Apoptosis of oligodendrocytes. Immunohistochemistry for oligodendrocyte marker MOSP (**a**) and apoptosis marker cleaved caspase-3 (Casp-3) (**b**) was performed. There were a lot of (c) MOSP- and Casp-3 double-positive apoptotic oligodendrocytes in injured spinal cord 1 week after SCI. In G-CSF-treated rats, the number of apoptotic oligodendrocytes decreased

significantly larger, and expression of angiogenic cytokines including bFGF, VEGF, and HGF was significantly higher than those in the control group. The G-CSF-treated group showed significant recovery of hind limb function compared to that of the control group. These results suggest that G-CSF exerts neuroprotective effects via promotion of angiogenesis after SCI [12].

13.2 Discussion

One of the major obstacles for conducting clinical trials for neuroprotective drugs is to first establish the safety and competency for use in human subjects. The complexity, size, and duration of clinical trials of novel drugs often make them quite costly to conduct and may impede the development of therapeutics that could have a significant impact in clinical practice. Therefore, although the efficacy of various drug therapies in models of SCI has been reported, few drugs have been practically carried into clinical trials. Thus, drugs with proven clinical exploitability have a significant advantage for clinical trials for novel therapeutic purposes. From this point of view, G-CSF's current use in the clinic for hematopoietic stimulation and its ongoing clinical trial for brain infarction make it an appealing molecule that could be rapidly placed into trials for acute SCI patients. Although many hurdles such as optimal dosage, therapeutic time window, and more precise mechanism of action still need to be resolved, the present results encourage us to make steps towards future clinical trials of G-CSF for acute SCI patients.

13.3 Conclusion

G-CSF exerts neuroprotective action for SCI via the abovementioned pleiotropic effects. Therefore G-CSF is one of the hopeful candidates for clinical application.

Acknowledgement Masao Koda declares that he has no conflict of interest.

References

1. Rabchevsky AG, Patel SP, Springer JE (2011) Pharmacological interventions for spinal cord injury: where do we stand? how might be we step forward? Pharmacol Ther 132:15–29
2. Matsumoto T, Tamaki T, Kawakami M et al (2001) Early complications of high-dose methylprednisolone sodium succinate treatment in the follow-up of acute cervical spinal cord injury. Spine 26:426–430
3. Nicola NA, Metcalf D, Matsumoto M et al (1983) Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. J Biol Chem 258:9017–9023
4. Roberts AW (2005) G-CSF: a key regulator of neutrophil production, but that's not all! Growth Factors 23:33–41
5. Harada M, Qin Y, Takano H et al (2005) G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 11:305–311
6. Schäbitz WR, Kollmar R, Schwaninger M et al (2003) Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. Stroke 34:745–751
7. Schneider A, Kruger C, Steigleder T et al (2005) The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. J Clin Invest 115:2083–2098
8. Shyu WC, Lin SZ, Lee CC et al (2006) Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. CMAJ 174:927–933
9. Koda M, Nishio Y, Kamada T, Someya Y, Okawa A et al (2007) Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice. Brain Res 1149:223–231
10. Nishio Y, Koda M, Kamada T, Someya Y, Kadota R et al (2007) Granulocyte colony-stimulating factor (G-CSF) attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. J Neuropathol Exp Neurol 66:724–731
11. Kadota R, Koda M, Kawabe J et al (2012) Granulocyte colony-stimulating factor (G-CSF) protects oligodendrocyte and promotes hindlimb functional recovery after spinal cord injury in rats. PLoS One 7(11):e50391. doi:10.1371
12. Kawabe J, Koda M, Hashimoto M et al (2011) Neuroprotective effects of granulocyte colony-stimulating factor and relationship to promotion of angiogenesis after spinal cord injury in rats: laboratory investigation. J Neurosurg Spine 15:414–421

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Case Report

Radiograms Obtained during Anterior Cervical Decompression and Fusion Can Mislead Surgeons into Performing Surgery at the Wrong Level

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A 68-year-old woman who suffered from C5 nerve palsy because of a C4-5 disc herniation was referred to our hospital. We conducted anterior cervical decompression and fusion (ACDF) at the C4-5 level. An intraoperative radiogram obtained after exposure of the vertebrae showed that the level at which we were going to perform surgery was exactly at the C4-5 level. After bone grafting and temporary plating, another radiogram was obtained to verify the correct placement of the plate and screws, and it appeared to show that the plate bridged the C5 and C6 vertebrae at the incorrect level. The surgeon was astonished and was about to begin decompression of the upper level. However, carefully double-checking the level with a C-arm image intensifier before additional decompression verified that the surgery was conducted correctly at C4-5. Cautiously double-checking the level of surgery with a C-arm image intensifier is recommended when intraoperative radiograms suggest surgery at the wrong level.

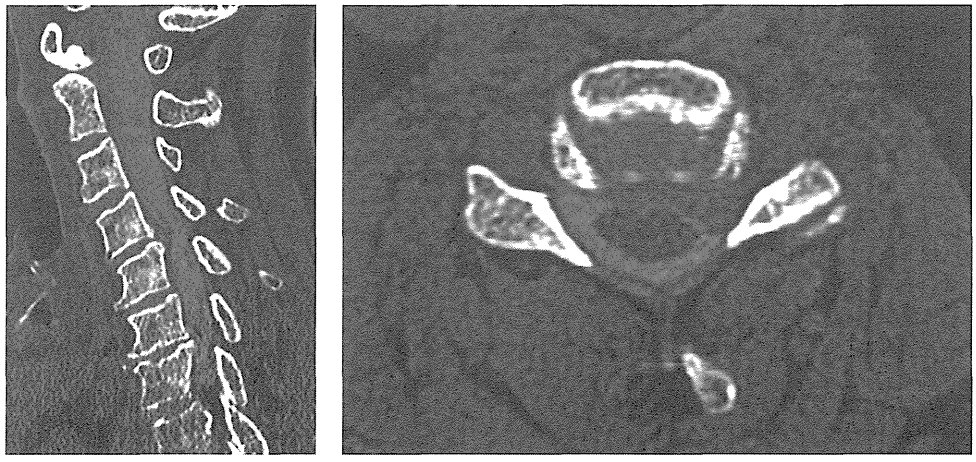
1. Introduction

Wrong-site surgery (WSS) is rare [1–15], but once it occurs, it distresses both patients and doctors [6]. Therefore, spine surgeons should make every effort to avoid wrong-site surgery. Here, we report a rare experience where a radiogram, which was obtained during anterior cervical decompression and fusion (ACDF), almost misled a surgeon into performing surgery at the wrong level.

2. Case Report

A 68-year-old woman suffered from left-side C5 nerve palsy because of a C4-5 disc herniation. Manual muscle testing scores of her left-side deltoid and biceps were 1 and 4, respectively, and physical examination showed no symptoms of myelopathy. Magnetic resonance imaging and computed tomography (CT) after myelography showed that the herniated disc at the C4-5 level compressed her left C5 nerve (Figure 1).

We conducted ACDF at the C4-5 level. During ACDF, we always obtain two radiograms to avoid WSS. One is taken after exposure of the vertebrae, with a needle inserted into a disc to verify that the level at which the decompression and fusion are to be conducted is correct. The other one is taken after temporary fixation of a plate following bone grafting to verify the correct placement of the plate and screws. During the surgery for the current case, the first radiogram showed that the needle was inserted into the C4-5 disc (Figure 2), so we continued the surgery and performed the herniotomy and bone grafting. After bone grafting, we positioned a plate to bridge the C4 and C5 vertebrae and fixed them temporarily. The radiogram after temporary placement of the plate astonished the surgeon because it appeared to show that the plate bridged the C5 and C6 vertebrae (Figure 3). The surgeon



(a) (b)

FIGURE 1: Computed tomography after myelography showing left-side C4-5 disc herniation. (a) Parasagittal view and (b) axial view at the C4-5 level.

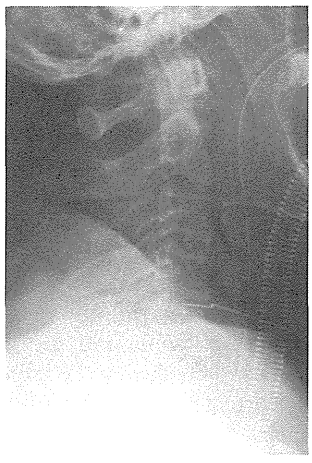


FIGURE 2: The first intraoperative radiogram after exposure of the vertebrae showing the needle inserted into the C4-5 disc.

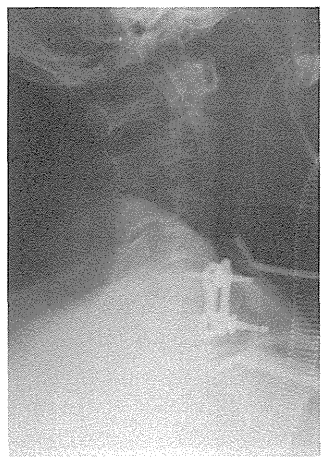


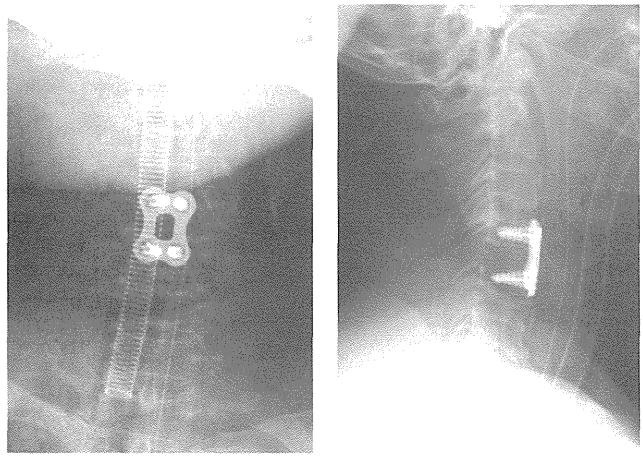
FIGURE 3: The second intraoperative radiogram after decompression, bone grafting and temporally plate fixation. It appears to show that the plate bridges the C5 and C6 vertebrae.

removed the plate and was about to begin decompression of the upper level. However, because we were unable to determine the reason why the level was apparently incorrect, we decided to double-check the level with a C-arm image intensifier before decompression of the upper level. The image verified that the surgery was conducted correctly at the level of C4-5, and not C5-6 as we were mistakenly led to believe. The final radiograms before the extubation also verified that the surgery was correctly performed at the C4-5 level (Figure 4).

After completing the surgery, we investigated why the radiogram apparently indicated the wrong site. Using a 3D CT image obtained after the surgery, we were able to construct a picture in which it appeared as if the plate bridged the C5 and C6 vertebrae (Figure 5). This revealed that the radiogram was taken from a caudal to cranial perspective during the surgery, and that the direction of exposure was not perpendicular to the axis of the spine.

3. Discussion

Various risk factors of WSS of the spine have been reported including emergency surgery, obesity, anatomic variations, time pressure to complete surgery, unusual equipment, multiple surgeons involved in the surgery, multiple procedures in a single surgery, and insufficient communication between the surgical team and the patient [10, 12, 14, 16–20]. In addition, failure to identify the vertebral level by intraoperative radiograms and misinterpretation of the radiogram are especially associated with wrong-level surgery [18, 20, 21]. As for cervical spine surgery, inadequate radiograms of the lower cervical spine hidden by the shoulders and cervical anomalies including Klippel-Feil syndrome and a block vertebra at C2-3 are major causes of wrong-level surgery [20]. In the current case, the patient did not have any of these factors.



(a) (b)

FIGURE 4: The final radiograms before extubation showed that ACDF was indeed performed at the correct level at C4-5. (a) Anteroposterior view and (b) lateral view.

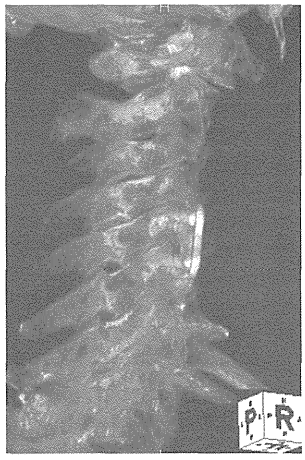


FIGURE 5: A constructed picture simulating the second intraoperative radiogram was obtained from 3D CT after the surgery. The plate appeared to bridge the C5 and C6 vertebrae.

There are some protocols for preventing WSS [22–24]. However, the effectiveness of the implementation of these protocols is controversial. Vachhani and Klopgenstein reported that the universal protocol (UP) by the Joint Commission on Accreditation of Healthcare Organizations was effective to reduce WSS events [25], but Wong and Watters III reported the UP was not effective [26]. Kwaan et al. reviewed cases and concluded that even the implementation of the UP would not have prevented 38% of WSS [8]. One of the main methods to avoid WSS is the use of radiograms during the surgery and this method is supported by many surgeons [6, 7, 14, 15, 27–29]. However, radiograms during the surgery cannot avoid every case of WSS because some patients have congenital anomaly of the spine or where radiograms are inadequate [10, 12, 25]. Some authors recommend using fluoroscopy during the surgery to identify correct levels for spinal surgery [13, 15, 30, 31]. Mayer et al. reported that surgeons now use fluoroscopy more frequently than plain radiograms during posterior surgery of the thoracic and lumbar spine, and surgeons who experienced WSS tend to have used plain radiograms more than fluoroscopy [31]. Intraoperative CT scan [32–36] is also useful to prevent WSS, but using this method routinely for only localizing the correct level is not practical.

In the current case, we obtained two radiograms during ACDF and the second radiogram almost misled a surgeon into performing unnecessary decompression at the wrong level, even though the patient did not have any anatomical anomalies of the cervical spine and the shoulders of the patient were pulled caudally during the radiograms to make it easier to see the correct level. On the other hand, a C-arm image intensifier clearly showed that we performed ACDF at the correct level. The cause of this event was that the second radiogram was inadequate and the surgeon could therefore not correctly interpret the picture. We constructed another picture from the 3D CT after the surgery that was similar to the second radiogram. This constructed picture revealed that the second radiogram was taken from a caudal to cranial perspective and the direction of exposure was not perpendicular to the axis of the spine as believed. Careful examination of Figure 5 shows that the "C3-4" disc is not clearly visualized. However, the surgeon in the operating room is under pressure to interpret radiograms quickly in less than ideal conditions and so their evaluation is compromised if they are inadequate. We highly recommend using a C-arm image intensifier to double-check the level of surgery if an intraoperative radiogram shows an unexpected finding, because a C-arm image intensifier can provide many images on many planes at once whereas plain radiograms do not offer real time feedback when the image is oblique or obscured by the shoulders. The fact that surgeons now use fluoroscopy more frequently than plain radiograms and surgeons who experienced WSS tend to have used plain radiograms more than fluoroscopy [31] also indicates that fluoroscopy is more useful than plain radiograms.

In conclusion, radiograms obtained during ACDF surgery can mislead surgeons into performing surgery at the wrong site. Cautiously double-checking the surgical level with a C-arm image intensifier is recommended when intraoperative radiograms suggest wrong-site surgery.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. W. Williams, "Microlumbar discectomy. A conservative surgical approach to the virgin herniated lumbar disc," *Spine*, vol. 3, no. 2, pp. 175–182, 1978.

[2] N. Eie, T. Solgaard, and H. Kleppe, "The knee-elbow position in lumbar disc surgery: a review of complications," *Spine*, vol. 8, no. 8, pp. 897–900, 1983.

[3] F. Ruggieri, L. Specchia, S. Sabalat, G. Galli, P. Ruggieri, and S. Arlecchini, "Lumbar disc herniation: diagnosis, surgical treatment, recurrence: a review of 872 operated cases," *Italian Journal of Orthopaedics and Traumatology*, vol. 14, no. 1, pp. 15–22, 1988.

[4] N. A. Ebraheim, C. Inzerillo, and R. Xu, "Are anatomic landmarks reliable in determination of fusion level in posterolateral lumbar fusion?" *Spine*, vol. 24, no. 10, pp. 973–974, 1999.

[5] E. G. Meinberg and P. J. Stern, "Incidence of wrong-site surgery among hand surgeons," *Journal of Bone and Joint Surgery*, vol. 85, no. 2, pp. 193–197, 2003.

[6] R. Goodkin and L. L. Laska, "Wrong disc space level surgery: medicolegal implications," *Surgical Neurology*, vol. 61, no. 4, pp. 323–342, 2004.

[7] J. M. Ammerman, M. D. Ammerman, J. Dambrosia, and B. J. Ammerman, "A prospective evaluation of the role for intraoperative x-ray in lumbar discectomy. Predictors of incorrect level exposure," *Surgical Neurology*, vol. 66, no. 5, pp. 470–473, 2006.

[8] M. R. Kwaan, D. M. Studdert, M. J. Zinner et al., "Incidence, patterns, and prevention of wrong-site surgery," *Archives of Surgery*, vol. 141, no. 4, pp. 353–358, 2006.

[9] S. C. Seiden and P. Barach, "Wrong-side/wrong-site, wrong-procedure, and wrong-patient adverse events: are they preventable?" *Archives of Surgery*, vol. 141, no. 9, pp. 931–939, 2006.

[10] B. S. Jhawar, D. Mitsis, and N. Duggal, "Wrong-sided and wrong-level neurosurgery: a national survey," *Journal of Neurosurgery: Spine*, vol. 7, no. 5, pp. 467–472, 2007.

[11] J. R. Clarke, J. Johnston, and E. D. Finley, "Getting surgery right," *Annals of Surgery*, vol. 246, no. 3, pp. 395–403, 2007.

[12] M. G. Mody, A. Nourbakhsh, D. L. Stahl, M. Gibbs, M. Alfawareh, and K. J. Garges, "The prevalence of wrong level surgery among spine surgeons," *Spine*, vol. 33, no. 2, pp. 194–198, 2008.

[13] J.-L. Pao, W.-C. Chen, and P.-Q. Chen, "Clinical outcomes of microendoscopic decompressive laminotomy for degenerative lumbar spinal stenosis," *European Spine Journal*, vol. 18, no. 5, pp. 672–678, 2009.

[14] J. Devine, N. Chutkan, D. C. Norvell, and J. R. Dettori, "Avoiding wrong site surgery: a systematic review," *Spine*, vol. 35, no. 9, pp. S28–S36, 2010.

[15] C. Irace and C. Corona, "How to avoid wrong-level and wrong-side errors in lumbar microdiscectomy: clinical article," *Journal of Neurosurgery: Spine*, vol. 12, no. 6, pp. 660–665, 2010.

[16] H. C. Pheasant, "Sources of failure in laminectomies," *The Orthopedic Clinics of North America*, vol. 6, no. 1, pp. 319–329, 1975.

[17] J. M. Ammerman and M. D. Ammerman, "Wrong-sided surgery," *Journal of Neurosurgery: Spine*, vol. 9, no. 1, pp. 105–106, 2008.

[18] M. A. James, J. G. Seiler III, J. J. Harrast, S. E. Emery, and S. Hurwitz, "The occurrence of wrong-site surgery self-reported by candidates for certification by the American Board of Orthopaedic Surgery," *Journal of Bone and Joint Surgery A*, vol. 94, no. 1, p. e2, 2012.

[19] M. W. Groff, J. E. Heller, E. A. Potts, P. V. Mummaneni, C. I. Shaffrey, and J. S. Smith, "A survey-based study of wrong-level lumbar spine surgery: the scope of the problem and current practices in place to help avoid these errors," *World Neurosurgery*, vol. 79, no. 3-4, pp. 585–592, 2013.

[20] M. A. Palumbo, A. J. Bianco, S. Esmende, and A. H. Daniels, "Wrong-site spine surgery," *Journal of the American Academy of Orthopaedic Surgeons*, vol. 21, no. 5, pp. 312–320, 2013.

[21] C. A. Fager, "Malpractice issues in neurological surgery," *Surgical Neurology*, vol. 65, no. 4, pp. 416–421, 2006.

[22] American Academy of Orthopedic Surgeons, *Information Statement WRONG-SITE SURGERY*, American Academy of Orthopedic Surgeons, Rosemont, Ill, USA, 1997, http://www.aaos.org/about/papers/advistmt/1015.asp.

[23] North American Spine Society, *Sign, Mark & X-ray (SMaX): prevent Wrong-Site Surgery*, North American Spine Society, La Grange, Ill, USA, 2014, http://www.spineline.org/Pages/PracticePolicy/ClinicalCare/SMAX/Default.aspx.

[24] Joint Commission on Accreditation of Healthcare Organizations, *Universal Protocol for Preventing Wrong Site, Wrong Procedure, Wrong Person Surgery*, Joint Commission on Accreditation of Healthcare Organizations, Oakbrook Terrace, Ill, USA, 2003, http://www.jointcommission.org/standards_information/up.aspx.

[25] J. A. Vachhani and J. D. Klopgenstein, "Incidence of neurosurgical wrong-site surgery before and after implementation of the universal protocol," *Neurosurgery*, vol. 72, no. 4, pp. 590–595, 2013.

[26] D. A. Wong and W. C. Watters III, "To err is human: quality and safety issues in spine care," *Spine*, vol. 32, no. 11, pp. S2–S8, 2007.

[27] R. K. Michaels, M. A. Makary, Y. Dahab et al., "Achieving the national quality forum's "never events": prevention of wrong site, wrong procedure, and wrong patient operations," *Annals of Surgery*, vol. 245, no. 4, pp. 526–532, 2007.

[28] U. G. Longo, M. Loppini, G. Romeo, N. Maffulli, and V. Denaro, "Errors of level in spinal surgery: an evidence-based systematic review," *Journal of Bone and Joint Surgery—Series B*, vol. 94, no. 11, pp. 1546–1550, 2012.

[29] W. Hsu, R. M. Kretzer, M. J. Dorsi, and Z. L. Gokaslan, "Strategies to avoid wrong-site surgery during spinal procedures," *Neurosurgical Focus*, vol. 31, no. 4, article E5, 2011.

[30] H. Singh, S. A. Meyer, A. C. Hecht, and A. L. Jenkins, "Novel fluoroscopic technique for localization at cervicothoracic levels," *Journal of Spinal Disorders and Techniques*, vol. 22, no. 8, pp. 615–618, 2009.

[31] J. Mayer, R. P. Dnag, G. F. D. Prieto, S. Qureshi, S. K. Cho, and A. Hecht, "Analysis of the techniques for thoracic and lumbar level localization during posterior spine surgery and the occurrence of wrong level surgery: results from a National survey," *The Spine Journal*, vol. 12, no. 9, pp. S71–S72, 2012.

[32] A. C. Hecht, S. M. Koehler, J. C. Laudone, A. Jenkins, and S. Qureshi, "Is intraoperative CT of posterior cervical spine instrumentation cost-effective and does it reduce complications?" *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 1035–1041, 2011.

[33] F. Costa, A. Cardia, A. Ortolina, G. Fabio, A. Zerbi, and M. Fornari, "Spinal navigation: standard preoperative versus intraoperative computed tomography data set acquisition for computer-guidance system: radiological and clinical study in 100 consecutive patients," *Spine*, vol. 36, no. 24, pp. 2094–2098, 2011.

[34] E. Ughwanogho, N. M. Patel, K. D. Baldwin, N. R. Sampson, and J. M. Flynn, "Computed tomography-guided navigation of thoracic pedicle screws for adolescent idiopathic scoliosis results in more accurate placement and less screw removal," *Spine*, vol. 37, no. 8, pp. E473–E478, 2012.

[35] G. Cui, Y. Wang, T.-H. Kao et al., "Application of intraoperative computed tomography with or without navigation system in surgical correction of spinal deformity: a preliminary result of 59 consecutive human cases," *Spine*, vol. 37, no. 10, pp. 891–900, 2012.

[36] J. K. Houten, R. Nasser, and N. Baxi, "Clinical assessment of percutaneous lumbar pedicle screw placement using the O-arm multidimensional surgical imaging system," *Neurosurgery*, vol. 70, no. 4, pp. 990–995, 2012.

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Clinical Study

Phosphorylated neurofilament subunit NF-H becomes elevated in the cerebrospinal fluid of patients with acutely worsening symptoms of compression myelopathy

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ABSTRACT

It is known that the severity of compression myelopathy sometimes worsens rapidly and results in poor functional recovery because of limited axonal regeneration. Levels of phosphorylated neurofilament subunit NF-H (pNF-H), which indicate axonal degeneration, are elevated in other neurological disorders. To our knowledge, there has been no examination of pNF-H levels in compression myelopathy. Therefore, we conducted a pilot cross-sectional study to evaluate pNF-H levels in the cerebrospinal fluid (CSF) of patients with worsening symptoms of cervical compression myelopathy. From January 2011 to March 2013, 51 samples of CSF were collected from patients at the time of myelography before spinal surgery. The indications for surgery were acutely worsening compression myelopathy (AM) in eight, chronic compression myelopathy (CM) in six, and lumbar canal stenosis (LCS) in 37 patients. The pNF-H levels were measured using a standard enzyme-linked immunosorbent assay. The mean ± standard deviation pNF-H value was 2127.1 ± 556.8 pg/ml in AM patients, 175.8 ± 67.38 pg/ml in CM patients and 518.7 ± 665.7 pg/ml in LCS patients. A significant increase in pNF-H levels was detected in the CSF of patients with AM compared with those with either CM or LCS. The clinical outcome of surgical treatment for patients with cervical myelopathy was satisfactory in both AM and CM patients. Despite the limitations of small sample size and lack of healthy CSF control data due to ethical considerations, our results suggest that pNF-H in CSF can act as a biomarker that reflects the severity of AM.

1. Introduction

Cervical compression myelopathy is one of the most common spinal cord disorders affecting the elderly. It is well known that the mechanism of compression myelopathy is chronic compression of the spinal cord by osteophytes, degenerated discs, thickened ligamenta flava, and ossification of the posterior longitudinal ligament [1]. Usually, a slow and stepwise decline in function is observed after compression myelopathy. However, a rapid progression of motor paralysis and paresthesia with mild or no trauma is occasionally observed. The severity of compression myelopathy has been reported to worsen rapidly in almost 5% of patients [2]. Rapid worsening of compression myelopathy results in severe neurological deficits with poor functional recovery because of limited axonal regeneration [1,3]. To date, the only effective therapy for compression myelopathy is early surgical treatment [4]. Generally, the recovery rate of neurological function after surgical treatment is about 50–70% [5]. However, in some patients, sufficient improvement of neurological function is not achieved. At present we cannot accurately predict the recovery rate before surgical treatment. Moreover, the only indicators to assess the severity of neurological status are subjective, including the Japanese Orthopaedic Association (JOA) score [6]. Therefore, biomarkers that reflect the degree of damage to the spinal cord and the severity of neurological symptoms would be useful.

Phosphorylated neurofilament subunit NF-H (pNF-H) is a structural protein of axon fibers and is not detected in the cerebrospinal

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fluid (CSF) or blood of healthy subjects. However, axonal breakdown increases the level of pNF-H in plasma and CSF [7]. A recent report has indicated that the level of pNF-H in the plasma and CSF is elevated in various neurological disorders such as subarachnoid hemorrhage, traumatic brain injury, amyotrophic lateral sclerosis, and acute spinal cord injury [8–12]. Therefore, pNF-H may be useful to evaluate the severity of progression and the effect of treatment in such disorders.

However, there are no studies examining the level of pNF-H in the CSF or plasma of patients with compression myelopathy to our knowledge. Therefore, we conducted a pilot cross-sectional study to determine the level of pNF-H in the CSF of patients with compression myelopathy.

2. Methods

2.1. Patients and samples

This study was given approval by our University Human Ethics Committee. From January 2011 to March 2013, 51 CSF samples were obtained from patients at the time of myelography before spinal surgery at the Toho University Sakura Medical Center. Informed consent was obtained from all patients. The indications for surgery were cervical compression myelopathy in 15 patients and lumbar canal stenosis (LCS), which was used as a control disorder, in 37 patients. Furthermore, we divided compression myelopathy samples into patients with acutely worsening symptoms (AM) and patients with chronic symptoms (CM). We defined acutely worsening compression myelopathy as that in which the JOA score of patients with cervical myelopathy decreased by 2 points or more during a recent 1 month period [13]. Ultimately, eight patients were allocated to the AM group and six patients to the CM group. Patients who were diagnosed as having cervical spondylotic radiculopathy and cervical spondylotic amyotrophy were excluded from this study. Patients with double lesions (cervical compression myelopathy and LCS) were also excluded.

2.2. Clinical outcome of patients with compression myelopathy

In all patients with compression myelopathy (AM and CM groups), neurological evaluation using a JOA score for cervical myelopathy (scores range from 0 to 17) was performed [6]. The scores were evaluated at the time of myelography before surgery and 6 months after surgery by two orthopedic spine surgeons.

2.3. pNF-H assay

The pNF-H assay was performed using a commercially available enzyme-linked immunosorbent assay kit (ELISA; BioVendor, Brno, Czech Republic). Frozen CSF samples were allowed to thaw, and diluted 1/2 in a buffer. The samples were then loaded onto an ELISA plate. The assay was performed according to the manufacturer's protocol. To standardize the pNF-H value, all samples were tested in duplicate, and the average value for each sample was calculated.

2.4. Statistical analyses

Results are presented as mean ± standard deviation. A one factor analysis of variance with a *post hoc* Tukey–Kramer test was used to evaluate the difference in the pNF-H levels between AM, CM, and LCS patients. Spearman's correlation coefficient by rank test was used to evaluate the correlation between pNF-H and JOA score. $p < 0.05$ was considered statistically significant.

Table 1
Patient characteristics in each group

	AM	CM	LCS
Patients, n	8	6	37
Sex			
Male	4	5	14
Female	4	1	23
Age, years*			
	64.9 ± 10.2 (45–79)	65.0 ± 13.2 (39–75)	70.3 ± 7.9 (55–86)
Preop JOA*	9.25 ± 2.43 (6–14)	10.6 ± 0.80 (10–12)	
Surgical procedure			
Laminoplasty	4	2	
Laminoplasty with posterior fusion	1	2	
Anterior corpectomy and fusion	3	2	

AM = acutely worsening compression myelopathy, CM = chronic compression myelopathy, JOA = Japanese Orthopaedic Association, LCS = lumbar canal stenosis, preop = preoperative.
* Data presented as mean ± standard deviation (range).

3. Results

3.1. Patient characteristics

Table 1 shows the characteristics of each group of patients. The mean age was 64.9 ± 10.2 (range 45–79 years) in the AM group, 65.0 ± 13.2 (range 39–75 years) in the CM group, and 70.3 ± 7.9 (range 55–86 years) in the LCS group. The mean JOA score at the time of CSF sampling in the AM group was 9.5 ± 2.51 (range 6–14), and 10.6 ± 0.80 (range 10–12) in the CM group. The surgical procedure in the AM group was laminoplasty in four patients, laminoplasty with posterior fusion in one patient, and anterior corpectomy and fusion in three patients. The surgical procedure in the CM group was laminoplasty in two patients, laminoplasty with posterior fusion in two patients, and anterior corpectomy and fusion in two patients.

3.2. Levels of pNF-H

Figure 1 shows the level of pNF-H in the CSF of patients from each group. The level of pNF-H was 2127.1 ± 556.8 pg/ml in the AM group, 175.8 ± 27.5 pg/ml in the CM group, and 518.7 ± 665.7 pg/ml in the LCS group. Our findings show that a significant increase in the level of pNF-H was detected in patients in the AM group compared with that in the CM and LCS group ($p < 0.01$). A slightly increased level of pNF-H was detected in CSF from patients in the LCS group compared with levels in the CM group. However, there was no significant difference in the levels between these two groups.

3.3. Evaluation of clinical outcome

Table 2 shows the change of JOA scores after surgery. JOA scores at the time of CSF collection were 9.5 ± 2.51 (range 6–14) in the AM group and 10.6 ± 0.80 (range 10–12) in the CM group. After surgery, neurological improvement was seen in all patients. JOA scores 6 months after surgery were 14.3 ± 1.82 (range 13.5–16.5) in the AM group and 13.9 ± 0.58 (range 13.5–15) in the CM group. The recovery rate of JOA score was 66.0 ± 16.9 (range 46.2–86.7) in the AM group and 51.2 ± 12.5 (range 30–66.7) in the CM group. Although a slightly higher recovery rate of JOA score was seen in the AM group, no statistical difference in recovery rate of JOA score was observed between patients in the AM and CM groups ($p = 0.096$).

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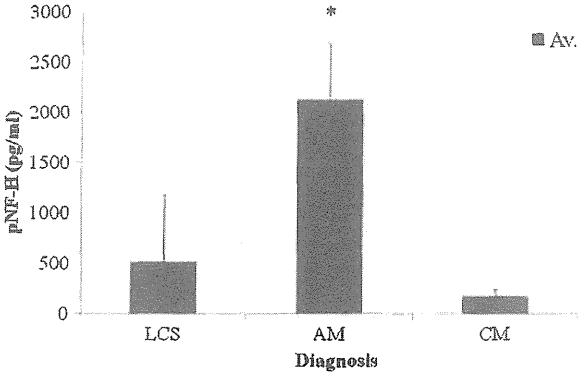

Fig. 1. Levels of phosphorylated neurofilament subunit NF-H. AM = acutely worsening compression myelopathy, Av. = average, CM = chronic compression myelopathy, LCS = lumbar canal stenosis. *$p < 0.01$ compared to lumbar canal stenosis and chronic compression myelopathy group.

Table 2
Recovery of Japanese Orthopaedic Association score

	AM	CM	*p* value
JOA score at the time of CSF collection	9.5 ± 2.51 (6–14)	10.6 ± 0.80 (10–12)	0.146
JOA score at 6 months after surgery	14.3 ± 1.82 (13.5–16.5)	13.9 ± 0.58 (13.5–15)	0.377
Recovery rate of JOA score	66.0 ± 16.9 (46.2–86.7)	51.2 ± 12.5 (30–66.7)	0.096

AM = acutely worsening compression myelopathy, CM = chronic compression myelopathy, CSF = cerebrospinal fluid, JOA = Japanese Orthopaedic Association.

No statistical correlation was found between the level of pNF-H and the recovery rate of JOA score ($p = 0.128$).

4. Discussion

To our knowledge, the present cross-sectional study is the first to determine the level of pNF-H in CSF samples from patients with cervical compression myelopathy and LCS. Our results showed a significant increase in the level of pNF-H of up to 2000 pg/ml in patients with AM. Elevated levels of pNF-H have suggested axonal breakdown in studies of other neurological disorders [7–9,11]. Furthermore, plasma pNF-H was found to be elevated proportional to the severity of acute spinal cord injury (SCI) and to reflect a greater extent of axonal damage because of the secondary damage to the injured spinal cord [10,12]. Increased levels of plasma pNF-H were seen in patients with complete SCI, but not in patients suffering incomplete paralysis [10]. In the present study, we hypothesized that increased levels of plasma pNF-H are not seen in compression myelopathy because of minor injury to the spinal cord compared with SCI. Therefore, we determined the levels of pNF-H in CSF rather than plasma. Although the pathogenesis and prognosis of compression myelopathy remain unclear, inflammation, hypoxia, and excitotoxicity are likely to cause secondary damage in SCI. An increase in the concentration of interleukin-6 has been detected in the CSF of patients with cervical compression myelopathy [14]. An increase in the concentration of interleukin-8 has been detected in the CSF of patients with cervical spondylotic myelopathy [15]. The increased level of pNF-H in the present study suggests that pNF-H reflects the severity of AM, and the pathogenesis in AM may be acute axonal damage followed by secondary damage, as seen in SCI.

In the present study, although a slightly higher recovery rate of JOA score was seen in the AM group, no statistical difference was observed between AM and CM patients. The surgical outcome was satisfactory in patients from both the AM and CM groups. There was no correlation between the level of pNF-H and the recovery rate of JOA score. Although surgical procedures for compression myelopathy are not standardized, our study suggests that early surgical treatment of AM results in sufficient neurological improvement, even in patients with CM.

The present study has several limitations. First, because CSF samples were only collected from patients at the time of myelography before surgery, the sample size was small and there is bias toward more severe disease. We found no statistical correlation between pNF-H and JOA recovery rate. However, a slightly higher JOA recovery rate was seen in AM patients. Further investigation with long-term follow-up after surgery and standardization of both the severity of the myelopathy and the surgical procedure performed are required to support our findings. Second, the collection method for CSF precludes the collection of CSF samples from healthy control subjects because of ethical issues. A slightly increased level of pNF-H was found in the CSF from LCS patients. A rodent study indicated that the level of pNF-is up-regulated in rat dorsal root ganglions [16]. In humans, increased interleukin-6 levels were detected in the CSF of patients with lumbar radiculopathy [14]. The present finding of slightly increased pNF-H levels in the CSF of patients with LCS may reflect axonal damage to the nerve roots or the cauda equina. The average level of pNF-H in the CSF of patients with LCS was about 500 pg/ml. The present findings suggest that pNF-H may be useful in the differential diagnosis of double lesions (cervical myelopathy and LCS). Further investigation using comparison samples from healthy control subjects is required. Third, the detailed pathogenesis of increased pNF-H levels in the CSF of patients with AM or CM remains unclear. Further research using animal models of compression myelopathy may clarify the pathogenesis.

In conclusion, despite the limitations indicated above, a significantly increased level of pNF-H was detected in the CSF of patients with AM. Clinical outcome after surgical treatment for cervical myelopathy was satisfactory in patients with both AM and CM. The present results suggest that pNF-H in CSF may be a biomarker that reflects the severity of AM.

Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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References

[1] Baba H, Maezawa Y, Imura S, et al. Quantitative analysis of the spinal cord motor neuron under chronic compression: an experimental observation. J Neurol 1996;243:109–16.
[2] Schmidt MH, Quinones-Hinojosa A, Rosenberg WS. Cervical myelopathy associated with degenerative spine disease and ossification of the posterior longitudinal ligament. Semin Neurol 2002;22:143–8.
[3] Fehlings MG, Skaf G. A review of the pathophysiology of cervical spondylotic myelopathy with insights for potential novel mechanisms drawn from traumatic spinal cord injury. Spine (Phila Pa 1976) 1998;23:2730–7.
[4] Sampath P, Benbebba M, Davis JD, et al. Outcome of patients treated for cervical myelopathy. A prospective multicenter study with independent clinical review. Spine (Phila Pa 1976) 2000;25:670–6.
[5] Zhu B, Xu Y, Liu X, et al. Anterior approach versus posterior approach for the treatment of multilevel cervical spondylotic myelopathy: a systemic review and meta-analysis. Eur Spine J 2013;22:1583–93.
[6] Masaki Y, Yamazaki M, Okawa A, et al. An analysis of factors causing poor surgical outcome in patients with cervical myelopathy due to ossification of

the posterior longitudinal ligament: anterior decompression with spinal fusion versus laminoplasty. J Spinal Disord Tech 2007;20:7–13.
[7] Shaw G, Yang C, Ellis R, et al. Hyperphosphorylated neurofilament NF-H is a serum biomarker of axonal injury. Biochem Biophys Res Commun 2005;336:1268–77.
[8] Anderson KJ, Scheff SW, Miller KM, et al. The phosphorylated axonal form of the neurofilament subunit NF-H (pNF-H) as a blood biomarker of traumatic brain injury. J Neurotrauma 2008;25:1079–85.
[9] Boylan K, Yang C, Crook J, et al. Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H) in blood of ALS model rodents and ALS patients: evaluation of blood pNF-H as a potential ALS biomarker. J Neurochem 2009;111:1182–91.
[10] Hayakawa K, Okazaki R, Ishii K, et al. Phosphorylated neurofilament subunit NF-H as a biomarker for evaluating the severity of spinal cord injury patients, a pilot study. Spinal Cord 2012;50:493–6.
[11] Lewis SB, Wolper RA, Miralia L, et al. Detection of phosphorylated NF-H in the cerebrospinal fluid and blood of aneurysmal subarachnoid hemorrhage patients. J Cereb Blood Flow Metab 2008;28:1261–71.
[12] Ueno T, Ohori Y, Ito J, et al. Hyperphosphorylated neurofilament NF-H as a biomarker of the efficacy of minocycline therapy for spinal cord injury. Spinal Cord 2011;49:333–6.
[13] Sakuma T, Yamazaki M, Okawa A, et al. Neuroprotective therapy using granulocyte colony-stimulating factor for patients with worsening symptoms of compression myelopathy, part 1: a phase I and IIa clinical trial. Eur Spine J 2012;21:482–9.
[14] Nagashima H, Morio Y, Yamane K, et al. Tumor necrosis factor-alpha, interleukin-1beta, and interleukin-6 in the cerebrospinal fluid of patients with cervical myelopathy and lumbar radiculopathy. Eur Spine J 2009;18:1946–50.
[15] Ito K, Matsuyama Y, Yukawa Y, et al. Analysis of interleukin-8, interleukin-10, and tumor necrosis factor-alpha in the cerebrospinal fluid of patients with cervical spondylotic myelopathy. J Spinal Disord Tech 2008;21:145–7.
[16] Jamieson SM, Jubramanian J, Liu JJ, et al. Oxaliplatin-induced loss of phosphorylated heavy neurofilament subunit neuronal immunoreactivity in rat DRG tissue. Mol Pain 2009;5:66.

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脊髄障害性疼痛に対する顆粒球コロニー刺激因子（G-CSF）の効果

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要　旨

われわれは，脊髄損傷に対する顆粒球コロニー刺激因子（G-CSF）の神経保護効果を基礎研究で報告し，2008年から実際に急性脊髄損傷，および病態が類似していると考えられる圧迫性脊髄症急性増悪に対する臨床試験を進めている．本試験において，予期せぬ効果として脊髄障害に起因する疼痛が軽減した症例を少なからず経験したことから，G-CSFの脊髄障害性疼痛に対する有効性が示唆された．今回，日常診療で治療に難渋することの多い，圧迫性脊髄症術後の脊髄障害性疼痛に対するG-CSFの臨床的効果と，基礎研究によるG-CSFの疼痛軽減機序について報告する．（ペインクリニック 35：1026-000，2014）

キーワード：脊髄障害性疼痛，顆粒球コロニー刺激因子，圧迫性脊髄症

はじめに

脊髄障害性疼痛とは2009年以降に本邦で使用されるようになった新しい概念で，何らかの脊髄障害に起因する痛みを総称している．神経障害性痛の関与が大きく，治療に難渋することが少なくない．本邦においては約半数の患者が圧迫性脊髄症に起因することが報告されており[1)]，手術による除痛効果も必ずしも芳しくない．そのため，現代の医学をもってしても十分な治療法が確立されていないといえる．

顆粒球コロニー刺激因子（Granulocyte-colony stimulating factor：G-CSF）は，顆粒球系細胞の分化・増殖・生存促進などの作用を有する造血性サイトカインで，本邦では，がん化学療法による好中球減少症や，末梢血幹細胞移植時の造血幹細胞の末梢血への動員などの目的で臨床使用されている[2)]．中枢神経系においては脳卒中モデルに対する神経保護作用が報告されており[3)]，脳梗塞に対する臨床試験も行われている[4)]ことから，われわれは脊髄損傷に対してもG-CSFが有効であると考え，動物実験モデルを用いてその有効性を報告してきた[5,6)]．また，Fehlingsら[7,8)]は，脊髄損傷と頸椎症性脊髄症の脊髄病変には類似点が多いため，脊髄損傷治療を目的として現在開発されている神経保護療法は，頸椎症性脊髄症に対しても応用可能であると報告している．この報告を踏まえ，われわれは2008年から，急性脊髄損傷および圧迫性

〈Special Article〉 Recent topics of the management of musculoskeletal pain
Therapeutic effects of granulocyte colony-stimulating factor (G-CSF) for spinal neuropathic pain
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表1　G-CSF投与前の患者背景

性　　別（男：女）〔症例〕	9：3
年　　齢〔歳〕（平均±SD）	69±6.5
診　　断：靱帯骨化症	8
その他	4
高　　位：頸　　椎	9
胸　　椎	3
罹病期間〔年〕（平均±SD）	7.9±8.5
疼痛部位：At-level	8
Below-level	8

脊髄症急性増悪に対し，G-CSFを用いた神経保護療法の臨床試験を進めている[9,10]．本試験において，G-CSFの安全性と神経保護効果が確認されたとともに，圧迫性脊髄症患者においては，予期せぬ効果として，脊髄障害に起因する疼痛が軽減した症例を少なからず経験した[11]．このことから，G-CSFの脊髄障害性疼痛に対する有効性が示唆されたため，圧迫性脊髄症に付随した脊髄障害性疼痛を対象に，G-CSFの臨床試験を行った[12]．今回は，日常診療で難渋することの多い，圧迫性脊髄症術後の脊髄障害性疼痛に対して行ったG-CSFの臨床試験結果と，その作用機序解明のために行った基礎研究について報告する．

1．圧迫性脊髄症術後の脊髄障害性疼痛に対する臨床試験

1）対象と方法

圧迫性脊髄症の術後症例で，術後半年以上経過しても疼痛が遺残して通常の内服治療（麻薬性鎮痛薬は使用していない）では十分な効果の得られない症例を対象とした．2009年8月から2011年10月の期間にG-CSFを投与し，6カ月以上経過観察し得たのは12症例であった．経過観察期間中には他の薬物の新規投与や物理治療等の開始は禁止した．投与量，期間は圧迫性脊髄症急性増悪症例のプロトコールと同じく10 μg/kg/日×5日間の点滴静注とした．

国際疼痛学会の分類に従い，疼痛部位と障害脊髄の位置関係から，脊髄障害性疼痛を障害髄節高位から2髄節以内の領域のAt-level painと3髄節以上尾側の領域のBelow-level painに分類した．疼痛の程度はvisual analogue scale（VAS：0～100）を用いて評価した．統計学的評価にはWilcoxonの符号付順位検定を用い，$p<0.05$を有意差ありとした．

2）結　　果

対象は，男性9症例，女性3症例で，平均年齢は69歳であった．半数以上が靱帯骨化症の症例で，障害脊髄高位は頸髄9症例，胸髄3症例であった．疼痛の罹病期間は平均7.9年と長かった．At-level painが4症例，Below-level painが4症例，両者の合併が4症例であった（表1）．事前に非ステロイド性抗炎症薬（NSAIDs）9症例，プレガバリン4症例，クロナゼパム2症例の他，1人平均2.6薬物を服用していた．副作用の問題や無効などの理由で，全く内服治療をしていない症例も2症例存在した．

難治性疼痛を反映して，G-CSF投与前のVAS値は全症例で50以上であった．G-CSF投与後に20以上のVAS値の改善が認められたのは12症例中7症例（58%）に留まり，そのうち6症例で投与後3カ月以降にVAS値の

表2　G-CSF 投与後の VAS の変化

症例 No.	VAS				
	投与直前	1週	1カ月	3カ月	6カ月
1	60	20	20	20	30
2	50	40	40	40	50
3	80	50	50	50	50
4	90	90	90	90	90
5	60	0	0	60	60
6	90	45	45	90	90
7	90	90	90	90	90
8	80	60	60	80	80
9	80	80	80	80	80
10	60	40	40	60	60
11	60	50	60	60	65
12	80	20	20	80	80
平均±SD	73±14	49*±28	50†±28	67‡±22	69‡±19

*：投与直前に比し著しく有意に減少（$p<0.01$）
†：投与直前に比し有意に減少（$p<0.05$）
‡：投与後1週に比し有意に増加（$p<0.05$）

再上昇を認めた（**表2**）．平均 VAS 値は投与前 73 から G-CSF 投与後1週で 49 と有意な改善が認められた（$p<0.01$）ものの，投与後3カ月では 67 と，投与前に近い状態に再増悪しており，疼痛軽減効果が失われていた（**図1**）．本試験において神経症状の改善はほとんど得られなかったが，重篤な有害事象の発生も認めなかった．

3）考　察

脊髄障害性疼痛は，本邦で提唱された新しい概念であり，まだ，未解明な点が多い．文献は，ほとんどが脊髄損傷後の神経障害性痛について報告されたものであり，現在の知見の多くはこれらの研究に基づいたものである．病態が複雑で未だ十分な機序の解明はされていないが，近年の研究により，疼痛の発現や遷延化の機序として，脊髄後角細胞の興奮性増加[13]，下行性疼痛抑制系の障害[14]，脊髄後角におけるグリア細胞の活性化[15]，脊髄後角での感覚伝達の長期増強（long-term potentiation）[16]，脊髄上位中枢の障害[17]などが解明されてきた．これらが複雑に関与して障害脊髄の後角レベル，あるいは上位中枢レベルに中枢性感作（central sensitization）や可塑的変化が生じることで，慢性疼痛に関与していると考えられている．

At-level pain は神経根や障害脊髄後角の障害を反映しており，末梢神経と中枢神経両方の関与が考えられる神経障害性痛である．脊髄損傷後，比較的短期間で出現するといわれている．一方で，Below-level pain は，At-level pain とは機序が異なり，脊髄視床路の障害を反映すると考えられており，純粋な中枢性神経障害性痛である．At-level pain より遅れて出現し，より難治性であることが指摘されている[18]．今回の検討では，罹病期間の長い術後遺残性疼痛のため難治性の Below-level pain の割合が多く，脊髄障害が索路にまで及んで感覚神経に可

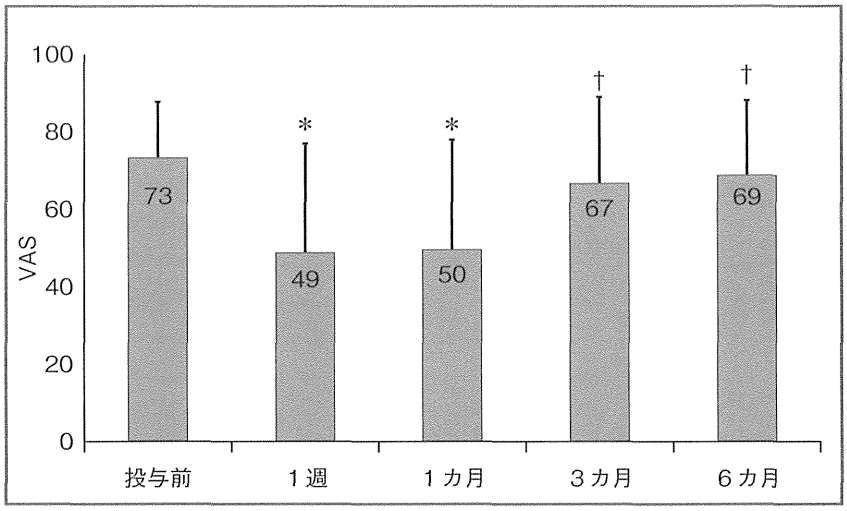

図1　G-CSF 投与前後平均 VAS 値
＊：p＜0.01（投与前比），†：p＜0.05（投与後1週比）

塑的変化が生じたため，G-CSF の効果が不十分な症例が多かった可能性が示唆される．

比較的研究の進んでいる脊髄損傷後神経障害性疼痛の薬物治療において，Baastrup らは，無作為化二重盲検試験の結果[19-22]から，抗てんかん薬であるプレガバリンとガバペンチン，三環系抗うつ薬であるアミトリプチリンの3薬物を第一選択薬として推奨している[23]．また，これらでも効果が不十分や，副作用等で使用できない場合には，セロトニン・ノルアドレナリン再取り込阻害薬（SNRI），トラマドール，オピオイドなども加え，多様な作用機序を組み合わせて治療していくことを重視している．しかし，これらのあらゆる薬物治療を行っても十分な効果が得られない症例も存在し，未だ適切な治療法が確立されていないのが現状である．

本邦では，脊髄障害性疼痛患者の約半数が圧迫性脊髄症に起因する[1]ため，脊髄損傷後神経障害性痛とは厳密には病態が異なる可能性があるが，圧迫性脊髄症に伴う疼痛に限局した報告となると極めて少なく，今までは脊髄症に対する効果判定として神経症状の改善ばかりに着目され，痛みについての評価がほとんどなされていなかったといえる．しかし，日常診療の場において，脊髄症は改善しても，痛みやしびれで苦しむ圧迫性脊髄症術後の患者を目にすることは少なくない．現在では，JOACMEQ などの患者立脚型の評価が使用されるようになってきており，患者の痛みが治療成績に評価されるようになった．また，平成22年度から，厚生労働省により「脊髄障害性疼痛症候群」が研究奨励分野に指定されて大規模研究が進められているため，今後は，圧迫性脊髄症に付随する痛みについての研究が進むことが期待される．数少ない報告として，竹下ら[24]は，圧迫性脊髄症の手術例と保存治療例で痛み，しびれの有病率は変わらないと報告している．また，橘ら[25]は，圧迫性脊髄症に伴う脊髄障害性疼痛は術後いったん改善するが，その後，再燃する傾向があると報告している．このことから，圧迫性脊髄症に伴う脊髄障害性疼痛に対する除圧手術の効果は限局的である可能性が示唆される．

今回の検討では，圧迫性脊髄症術後の遺残性疼痛における G-CSF の効果は，全症例に満足