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Background and Purpose. The efficacy of task-specific gait training for people with spinal cord injury (SCI) is premised on evidence that the provision of gait-related afferent feedback is key for the recovery of stepping movements. Recent findings have shown that sensory feedback from flexor muscle afferents can facilitate flexor muscle activity during the swing phase of walking. This case report was undertaken to determine the feasibility of using robot-applied forces to resist leg movements during body-weight–supported treadmill training (BWSTT) and to measure its effect on gait and other health-related outcomes.

Case Description. The patient described in this case report was a 43-year-old man with a T11 incomplete chronic SCI. He underwent 36 sessions of BWSTT using a robotic gait orthosis to provide forces that resist hip and knee flexion.

Outcomes. Tolerance to the training program was monitored using the Borg CR10 scale and heart rate and blood pressure changes during each training session. Outcome measures (ie, 10-Meter Walk Test, Six-Minute Walk Test, modified Emory Functional Ambulation Profile [mEFAP], Activities-specific Balance Confidence Scale, and Canadian Occupational Performance Measure) were completed and kinematic parameters of gait, lower-extremity muscle strength (force-generating capacity), lower-limb girth, and tolerance to orthostatic stress were measured before and after the training program.

Discussion. The patient could tolerate the training. Overground walking speed, endurance, and performance on all subtasks of the mEFAP improved and were accompanied by increased lower-limb joint flexion and toe clearance during gait. The patient’s ambulatory self-confidence and self-perceived performance in walking also improved. These findings suggest that this new approach to BWSTT is a feasible and potentially effective therapy for improving skilled overground walking performance.
Body-weight–supported treadmill training (BWSTT) is a rehabilitation intervention used to promote the recovery of walking in individuals with motor-incomplete spinal cord injury (SCI). This intervention is based on the concept that the provision of appropriate (gait-related) afferent feedback during training is critical to initiating neuroplastic changes associated with improved walking following injury.1

Afferent feedback from muscle receptors contributes to extensor muscle activity during the stance phase of locomotion.2 During BWSTT, partial body-weight support (BWS) permits lower-limb loading while maintaining upright posture.3,4 Improvements in motor output associated with this type of training are exemplified by data showing that the progressive reduction in BWS over the course of training is accompanied by improved locomotor function3,5,6 and greater extensor muscle activity during stance.5

Body-weight–supported treadmill training strategies have relied on therapists or robotic devices to assist leg movements and ensure adequate foot clearance during the swing phase.4,6 Thus, in contrast to the sensory feedback provided to the extensor muscles during stance, afferent feedback to the flexor muscles is diminished as they are effectively unloaded and assisted through swing. Experiments in cats have shown that assisting hip flexion during swing reduces flexor burst activity, whereas the opposite occurs when hip flexion is slowed or blocked during swing.7 Evidence from studies that used direct stimulation of the nerves supplying the flexor muscles indicates that this effect could be mediated by sensory signals from group I flexor muscle afferents.8,9 Resisting limb flexion during swing would slow the rate of contraction and increase the load on the flexor muscles. These changes would enhance activation of length- and load-sensitive receptors in the flexor muscles, which, in turn, could sustain excitatory drive to flexor motoneurons through feedback pathways.8,9 The opposite would occur when the limb is assisted through flexion, whereby the flexor muscles would undergo unloading and length changes more rapidly than usual, prematurely decreasing excitatory drive to flexor motoneurons during locomotion.7

Findings in human infants and adults who are able-bodied and in individuals with motor-incomplete SCI are consistent with this model of sensory feedback modulation of flexor muscle activation during swing. Immediate increases in flexor muscle activity were observed with the addition of leg weights around the lower limbs of human infants10 and adults11 who were able-bodied and in individuals with motor-incomplete SCI.12 The application of robotic forces to resist the hip and knee during swing also elicited increases in flexor muscle activity.13,14 These findings are consistent with the concept that transmission through muscle afferent feedback pathways could contribute to flexor muscle activity during walking in humans.

During swing, adequate flexor muscle activation is important for foot clearance. Following a program of BWSTT, Grasso et al15 found that the toe trajectory during treadmill locomotion in people with subacute SCI recovered to within the range of control values. However, this recovery was accompanied by excessive reliance on activity from proximal limb and trunk muscles. In another study of people with chronic SCI, increases in tibialis anterior and hamstring muscle activity during treadmill locomotion were associated with improved ambulatory capacity following BWSTT.16 However, there were no data on how those changes in muscle activity might have translated to the quality of the kinematic gait pattern during overground walking. Furthermore, although toe trajectory patterns during treadmill locomotion may recover,19 there are many more-complex overground gait skills (eg, obstacle clearance, stair climbing) in which the ability to lift the leg beyond a certain height is essential.

Improvements in lower-limb muscle cross-sectional area and strength (force-generating capacity) have been shown following either BWSTT17,18 or resistance training.19 Body-weight–supported treadmill training can improve the autonomic regulation of heart rate (HR) and blood pressure (BP) and has a positive effect on vascular dynamics.20 These are key findings considering the prevalence of deconditioning and decreased cardiovascular fitness in individuals with SCI.21,22 Given the potential wide-ranging health benefits of locomotor training, the assessment of the effects of new interventions should be comprehensive and include measures beyond ambulatory outcomes.

The purpose of this case report is to describe a novel approach to BWSTT augmented with forces to resist leg movements during gait. We anticipated that the application of forces that can resist leg flexion during the swing phase could help to strengthen motor patterns during swing. Tolerance to BWSTT with robot-applied
resistance was assessed by tracking the level of perceived exertion and cardiovascular tolerance during training. The effect of this intervention was assessed by measures of over-ground walking speed, distance, and skilled walking, as well as muscle strength, limb girth, confidence in and satisfaction with walking performance, and changes in response to orthostatic challenge.

Patient History and Review of Systems

The patient was a 43-year-old man (height = 180 cm, weight = 77 kg). He incurred a traumatic SCI due to a T12 burst fracture following a fall 2 years prior to the intervention described in this case report. He was completely independent with activities of daily living, primarily using a manual wheelchair for indoor and outdoor mobility, but was able to stand and walk indoors for brief periods during the day.

Clinical Impression

The patient’s limited ambulatory capacity combined with his residual motor function made him an ideal candidate for this intervention. The patient provided written informed consent. All procedures were approved by the University of British Columbia Clinical Research Ethics Board.

Examination

Neurologic examination established that this individual had sustained a T11 American Spinal Injury Association Impairment Scale25 D SCI (with mixed conus medullaris and cauda equina injury). His total Lower Extremity Motor Score (LEMS) was 29/50, with no visible or palpable contraction in the right L4, L5, and S1 motor segments (Tab. 1). Sensory examination revealed that the last normal sensory level was T11. There was preservation of the deep anal sensation.

Table 1.

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>Right Side</th>
<th>Left Side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Posttraining</td>
</tr>
<tr>
<td>Key muscles*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip flexors</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Knee extenders</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Big toe extenders</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ankle plantar flexors</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee flexors</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hip extenders</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hip abductors</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hip adductors</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* According to the American Spinal Injury Association Impairment Scale.

The patient reported occasional episodes of dizziness or light-headedness, but could not associate these symptoms with any specific event. His reflex response to orthostatic challenge was assessed using the Sit-Up and Stand-Up Test.24 Resting BP was within the normal range (130/80 mm Hg), and there was no orthostatic decrease in arterial BP during the orthostatic challenge test. His HR response, however, showed mild tachycardia in response to the stress of sitting (from 89 bpm at rest to 106 bpm at 3 minutes after sit-up), with no obvious decline in BP.

Clinical Impression

The patient had sufficient lower-limb strength to undergo the intervention. His BP and HR responses to the Sit-Up and Stand-Up Test were consistent with possible deconditioning.

Intervention

The BWSTT was implemented using customized software control of the Lokomat robotic gait trainer. The drives were programmed to apply a velocity-dependent moment against sagittal-plane hip and knee movements throughout the gait cycle. Movements along the frontal and transverse planes were restricted by the structure of the Lokomat. The instantaneous torques (\( \mathbf{M} \)) applied to the hip (H) and knee (K) were defined by:

\[
\mathbf{M} = \begin{bmatrix}
B_H & 0 \\
0 & B_K
\end{bmatrix}
\begin{bmatrix}
\dot{\theta}_H \\
\dot{\theta}_K
\end{bmatrix}
\]

where \( B_H \) and \( B_K \) are the corresponding viscous coefficients (N \( \cdot \) m \( \cdot \) s/rad) and \( \dot{\theta}_H \) and \( \dot{\theta}_K \) are the angular velocities (rad/s) of the hip and knee, respectively. Although the resistance was applied throughout the gait cycle, we expected the effect of the resistance to be greatest during the swing phase. Resistance applied during the stance phase will be assisted by the movement of the treadmill belt, whereas there is no means to assist swing phase motion against the resistance. Unfortunately, we did not record lower-limb kinematics during the actual training sessions of this patient. However, data are presented from 3 individuals who participated in a related study (unpublished results) in which the same type of resistance was used (Fig. 1).
These data show the hip and knee joint kinematics during a training session and the accompanying pattern of added resistance throughout the gait cycle. The amount of resistance was based on maximum voluntary contraction (MVC) testing and the patient’s current training speed. First, the patient performed isometric strength testing using the Lokomat’s “L-Force” feature. Three trials were performed to calculate an average MVC for the hip and knee flexors bilaterally. Second, the patient walked with the Lokomat at his previous treadmill training speed (for the first training session, treadmill speed was set at 1.1 km/h, which was his initial preferred training speed with the Lokomat). During these trials, the electromechanical drives of the Lokomat were controlled, using a model-based impedance control algorithm, to compensate for the gravitational, Coriolis, and friction effects resulting from the orthosis. The Lokomat controller minimizes any resistance or assistance to leg movements during walking (null field walking). The patient reported that he was able to step of his own volition. We conducted a previous study in individuals without disabilities and showed that muscle activity and lower-limb kinematics during null field walking are similar to what would be expected during normal treadmill walking. Data were recorded for approximately 1 minute during this null field trial, and analog signals from the Lokomat’s position sensors at the hip and knee were recorded at 1,000 Hz to a personal computer. Using a custom-written MATLAB routine, the hip and knee angular velocity during swing (defined by the time between the onsets of hip flexion and extension) were determined. The average angular velocities of the hip and knee during swing (\(\dot{\theta}_H\) and \(\dot{\theta}_K\)) then were used to determine the desired \(B\) values for the hip and knee to be used during training. \(M_H\) and \(M_K\) were defined as 5% of MVC. The desired \(B\) values were reassessed in this way every 4 to 6 training sessions.

The level of BWS was adjusted to the minimum tolerated by the patient while ensuring appropriate stance-phase kinematics. In the first training session, the patient was provided with 50% of BWS. Each subsequent training session was initiated at the lowest level of BWS provided during the previous training session. We observed the quality of the patient’s knee joint kinematics during stance as a measure of his tolerance to a given level of BWS. If the knee buckled during stance, we increased the amount of BWS by 5 kg. If the patient was able to step with appropriate stance-phase kinematics at a given level of BWS for at least 5 minutes, we lowered the amount of BWS by 5 kg.

Treadmill speed was set to the fastest speed that the patient could tolerate. In the first training session, the speed was 1.1 km/h. Each subsequent training session started with a warm-up for 3 to 5 minutes at the lowest speed used in the previous training session. Treadmill speed subsequently was increased by increments of 0.1 km/h. The patient’s tolerance to a given speed was evaluated by observing his ability to advance his feet beyond his hips. If he was able to keep up with the treadmill speed for at least 15 minutes, another increment of 0.1 km/h was added. Because the patient had no active control over his right ankle, we used passive foot lifters to maintain it in a neutral position throughout training.

Three training sessions per week for a total of 12 weeks were planned. Each session consisted of 45 minutes of treadmill walking (not including rest breaks).
Outcomes
Tolerance to Training
The patient’s level of perceived exertion was tracked using the Borg CR10 scale.27 He rated his level of perceived exertion with respect to the sense of effort in the leg muscles and in the cardiorespiratory system. The Borg CR10 scale was administered once the treadmill came to a stop at the beginning of each rest break (approximately every 10 minutes). We also monitored his HR and oxygen saturation throughout training, and BP measurements were taken during each rest break using a portable vital signs monitoring system (Carescape V100).27

Ambulatory Capacity
Changes in the patient’s ambulatory capacity were assessed using the 10-Meter Walk Test (10MWT) and Six-Minute Walk Test (6MWT). Both measures have excellent test-retest reliability (r = .985 and .981, respectively) in people with SCI.28 Both measures also show good validity with the Timed “Up & Go” Test (TUG) (r = .89 and .88, respectively) and each other (r = -.95).28 The patient’s dependence on ambulatory aids was assessed using the Walking Index for Spinal Cord Injury (WISCI).29

We recorded the patient’s ability to perform a variety of skilled walking tasks using the modified Emory Functional Ambulation Profile (mEFAP),30 which records the time and amount of assistance required to complete 5 tasks: (1) walking on a smooth floor surface for 5 m; (2) walking on a low-pile carpet for 5 m; (3) rising from a chair, walking 3 m, and returning to sit in the chair (TUG); (4) obstacle avoidance; and (5) stair climbing (75-cm stair width and 15-cm stair height). The mEFAP is reliable (test-retest intraclass correlation coefficients > .97)30,31 and has good validity with the 10MWT (r = .88-.93).31 We also recorded the time required for the patient to walk up and down a 1.2-m, 5-degree ramp.

Lower-limb kinematics during straight, overground walking (over a 5-m walkway) and stair climbing were recorded before and after training. Markers were placed over the following landmarks on the toe, ankle, knee, and hip: fifth metatarsal head, lateral malleolus, lateral femoral condyle, and greater trochanter. Kinematic data were recorded by position sensors at 100 Hz (Optotrak). Offline data processing was performed using MATLAB. All signals were low-pass filtered at 6 Hz using a digital, zero-lag, fourth-order Butterworth filter. Changes in step length, toe trajectory height, and peak hip and knee flexion during swing and joint excursion over the whole gait cycle were compared before and after training. Toe clearance over the stairs was measured as the vertical distance between the edge of each step and the fifth metatarsal head.

Muscle Strength and Lower-Limb Girth
Lower-limb strength was assessed using the LEMS. Manual muscle testing also was used to track changes in other leg muscles (Tab. 1). The circumference of the leg 15 cm above the superior border of the patella was used as a measure of thigh girth. The circumference of the leg 20 cm below the inferior border of the patella was used to measure shank girth.

Cardiovascular Tolerance
The change in the patient’s cardiovascular tolerance was tracked by evaluation of his baseline cardiovascular parameters and reflex changes due to orthostatic challenge (Sit-Up and Stand-Up Test). Tolerance was tracked by recording changes in resting HR and BP during these orthostatic challenge tasks.24

Confidence in and Satisfaction With Walking Performance
Ambulatory self-confidence and self-perceived change in walking performance were assessed using the Activities-specific Balance Confidence Scale (ABC)32 and the Canadian Occupational Performance Measure (COPM),33 respectively. The ABC is a 22-item questionnaire that asks individuals to rate their self-confidence in performing a variety of ambulation tasks. The COPM involved a semistructured interview with the participant to determine his self-identified areas of difficulty related to walking. He was asked to identify his top problem areas related to ambulatory capacity and to rate them on a scale of 1 to 10 with respect to his perceived level of performance and satisfaction (score of 10 refers to highest level of performance or satisfaction).

Training Intervention
The patient completed 36 training sessions. He initially trained at 50% of BWS, which was reduced to 30% to 40% of BWS at week 3 and maintained at this level for the remainder of the training program. The initial treadmill speed was set at 1.1 km/h. The treadmill speed was increased to 1.8 km/h by the sixth week of training and remained at that speed for the remainder of the training program. The change in BWS and treadmill speed parameters is consistent with the findings of a previous clinical study of Lokomat-assisted BWSTT.34 Table 2 contains the average MVC values recorded by the Lokomat throughout the training program to determine the amount of added resistance (B values). At the beginning of the training program, the average

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1 GE Healthcare UK Ltd, Amersham Place, Pollards Wood, Nightingales Lane, Chalfont, St Giles HP8 4SP, United Kingdom.

2 Northern Digital Inc, 103 Randall Dr, Waterloo, Ontario, Canada N2V 1C5.
amount of added resistance against right and left hip movements was 2.4 and 3.7 Nm, respectively. By the end of training, this amount had increased to 2.8 and 4.5 Nm, respectively. The average amount of added resistance to the left knee increased from 0.6 to 0.9 Nm by the end of training. Very little resistance (0.1 Nm) was applied against right knee movements over the course of the training program.

Tolerance to training. The patient’s self-reported Borg CR10 scale score with respect to feelings of exertion in his legs ranged from 4 (“somewhat strong”) to 7 (“very strong”) over the course of training. His self-reported feelings of exertion with respect to his cardiorespiratory system ranged from 3 (“moderate”) to 5 (“strong/heavy”). Maximum HR over the duration of the training program never exceeded 122 bpm, and average oxygen saturation ranged from 94% to 98%. Mean arterial pressure ranged from 75 to 113 mm Hg over the duration of training. The patient had no adverse events related to the training.

Ambulatory capacity. The patient’s 10MWT scores improved from 0.18 m/s to 0.25 m/s (+36.8%), and his 6MWT scores improved from 58.8 m to 79.9 m (+35.9%) following training. The WISCI scores remained constant at 13 (walking with a walker). The patient’s performance on skilled walking tasks also improved (Tab. 3).

Figure 2 illustrates the changes in gait kinematics during straight over-ground walking (Figs. 2A and 2B) and stair climbing (Fig. 2C). Qualitatively, the patient’s walking pattern improved, with increased joint excursions and toe clearance height. Improvements were noted in almost all kinematic variables for both the right and left legs (Tab. 4).

**Table 2.** Maximum Voluntary Contraction (Nm)

<table>
<thead>
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<th>Training Session</th>
<th>Right Side</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hip Flexion</td>
<td>Knee Flexion</td>
</tr>
<tr>
<td>1</td>
<td>48.1</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>53.2</td>
<td>2.4</td>
</tr>
<tr>
<td>11</td>
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<td>1.0</td>
</tr>
<tr>
<td>17</td>
<td>55.9</td>
<td>1.6</td>
</tr>
<tr>
<td>22</td>
<td>56.7</td>
<td>1.4</td>
</tr>
<tr>
<td>29</td>
<td>55.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Table 3.** Change in Skilled Walking Capacity

<table>
<thead>
<tr>
<th>Task</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-m smooth floor (s)</td>
<td>33.40°</td>
<td>17.42°</td>
<td>-15.98</td>
</tr>
<tr>
<td>5-m carpet (s)</td>
<td>28.86°</td>
<td>23.23°</td>
<td>-5.63</td>
</tr>
<tr>
<td>Timed Up and Go (s)</td>
<td>69.40°</td>
<td>43.00°</td>
<td>-26.40</td>
</tr>
<tr>
<td>Obstacles (s)</td>
<td>132.38°</td>
<td>79.99°</td>
<td>-52.39</td>
</tr>
<tr>
<td>Stairs (s)</td>
<td>41.46°</td>
<td>24.20°</td>
<td>-17.26</td>
</tr>
<tr>
<td>Ramp (s)</td>
<td>44.57°</td>
<td>32.52°</td>
<td>-12.05</td>
</tr>
<tr>
<td>Total mEFAP score without assistance factor (s)</td>
<td>305.50</td>
<td>187.84</td>
<td>-117.66</td>
</tr>
<tr>
<td>Total mEFAP score with assistance factor</td>
<td>5,499</td>
<td>2,254</td>
<td>-3,245</td>
</tr>
</tbody>
</table>

* TUG = Timed “Up & Go” Test, mEFAP = modified Emory Functional Ambulation Profile.
* Negative value denotes an improvement.
* Independent + walker + ankle-foot orthosis.
* Hit all 4 obstacles.
* This task is not included in the total mEFAP score.

**Discussion**

In this case report, we combined BWSTT with resistance training using Lokomat-applied forces. Our
Figure 2.
Changes in gait kinematics following training. (A) Stick figure reconstructions of the patient’s right leg during 1 overground step. (B) Averaged joint angles of the right and left hip, knee, and ankle during a step cycle. Upward deflections represent flexion. All data were normalized in time to 100% of the step cycle. (C) Right toe trajectory (using the marker on the right fifth metatarsal head) during 1 climb up a set of 4 steps. Gray lines represent pretraining data, and black lines represent posttraining data.
findings demonstrate that this is a feasible and potentially beneficial approach to improving functional ambulation in people with chronic motor-incomplete SCI. Not only did the patient demonstrate improvements in general indicators of overground ambulatory capacity, but he also exhibited improved functional capacity in more-complex tasks, notably obstacle clearance, stair climbing, and walking up a ramp. Associated with the functional improvements were increased lower-limb joint excursion and toe clearance during gait, as well as improved confidence and self-perceived performance and satisfaction with mobility tasks. In addition, the data indicated improvements in cardiovascular tolerance to orthostatic stress.

Although the patient underwent only treadmill-based locomotor training, improvements in walking capacity generalized to overground ambulatory skills that were not specifically trained. For example, average toe clearance over the stairs improved from 4 to 11 cm. Other researchers have shown that the average toe clearance height over stairs of a similar rise in adult controls is 20 cm. In addition, the patient improved his ability to step over obstacles. Recently, skilled walking training, comprising explicit practice of various overground gait skills, was introduced as another form of gait training for people with SCI. One of the hypothesized benefits of overground gait training is that it challenges dynamic balance control during functional gait tasks. Participants in that study showed improvements in both mEFAP scores and ambulatory self-confidence. Our patient did not undertake explicit practice of any specific gait skills but showed improvements in mEFAP and ABC scores that were within the range of those reported following skilled overground walking training.

We also noted other benefits of this training program that are consistent with previous findings. Although there was no obvious decline in arterial blood pressure during the orthostatic challenge in this individual, he exhibited significant tachycardia with standing prior to training. This outcome could be the result of deconditioning. These abnormal cardiovascular responses were normalized following training.

A limitation of this case report is that only 1 patient was tested and thus the findings cannot be generalized to the larger SCI population. We also relied on clinical gait observation to ensure that leg kinematics during training were appropriate. In future studies, computerized joint kinematic recordings should be used to quantify and confirm gait kinematics during training. In addition, we cannot discount the possibility that BWSTT alone (without the resistance) could have yielded the same results. Indeed, the improvements in overground walking speed recorded here were within a range similar to those of a previous study that used the Lokomat for BWSTT. Notably, however, the observed improvements in tasks requiring adequate swing phase activity (eg, stair climbing, obstacle clearance) were consistent with the conceptual framework underlying the rationale for using resistance. To date, there has been little information about how these more-complex gait tasks are influenced by BWSTT.

In summary, this case report has shown the feasibility of implementing a novel BWSTT strategy using robot-applied resistance against leg movements. Improvements in walking generalized not only to level, overground walking but also to more-skilled gait tasks.

Dr Lam provided concept and idea. Dr Lam and Dr Eng provided project design. Dr Lam, Dr Krassioukov, and Dr Eng provided writing. Dr Lam, Ms Pauhl, and Dr Krassioukov provided data collection. Dr Lam and Dr Krassioukov provided data analysis. Dr Lam and Ms Pauhl provided project management. Dr Lam provided fund procurement.
the patient, facilities/equipment, and institutional liaisons. Ms Pauhl provided consultation (including review of manuscript before submission). The authors are grateful to the patient for his commitment to this case report. The authors also thank Jennifer Loffree for her valuable assistance during this project.

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