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CASE REPORT

Combined Ankle Robot Training and Robot-assisted Gait Training Improved the Gait Pattern of a Patient with Chronic Traumatic Brain Injury

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Background: Walking disability caused by central nervous system injury often lingers. In the chronic phase, there is great need to improve walking speed and gait, even for patients who walk independently. Robot-assisted gait training (RAGT) has been widely used, but few studies have focused on improving gait patterns, and its effectiveness for motor function has been limited. This report describes the combination of "RAGT to learn the gait pattern" and "ankle robot training to improve motor function" in a patient with chronic stage brain injury. **Case**: A 34-year-old woman suffered a traumatic brain injury 5 years ago. She had residual right hemiplegia [Fugl-Meyer Assessment-Lower Extremity (FMA-LE): 18 points] and mild sensory impairment, but she walked independently with a short leg brace and a cane. Her comfortable gait speed was 0.57 m/s without an orthosis, and her 6-m walk test distance was 240 m. The Gait Assessment and Intervention Tool (G.A.I.T.) score was 35 points. After hospitalization, ankle robot training was performed daily, with RAGT performed 10 times in total. Post-intervention evaluation performed on Day 28 showed: FMA-LE, 23 points; comfortable walking speed, 0.69 m/s; G.A.I.T., 27 points; and three-dimensional motion analysis showed ankle dorsiflexion improved from 3.22° to 12.59° and knee flexion improved from 1.75° to 16.54° in the swing phase. **Discussion**: This is one of few studies to have examined the combination of two robots. Combining the features of each robot improved the gait pattern and motor function, even in the chronic phase.

Key Words: chronic; gait rehabilitation; HAL; RAGT; traumatic brain injury

INTRODUCTION

Traumatic brain injury remains a major contributor to physical impairment and loss of productivity, with a reported 2.3 million cases in the United States in 2014.[1] Injuries to the central nervous system (CNS), including stroke and traumatic brain injury, cause gait disturbances such as decreased walking speed and endurance.[2] Even mild traumatic brain injuries may cause long-term gait disturbances in up to 30% of patients.[3] As a result, reacquisition of normal gait is an important goal for many patients after CNS injury.[4] In many cases of traumatic brain injury, neurological recovery plateaus after about 6 months.[5] However, patients in the chronic phase (those that have had the disability for more than 6 months) have moved into the living phase and are living with their disability. To improve social activities and quality of life during the living phase, even if the patient can walk independently, it is necessary to improve gait endur-

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ance and gait efficiency.[6]

Gait rehabilitation has incorporated a variety of techniques, but since the 2000s, robotic rehabilitation has rapidly become popular. The use of robots provides repetitive, high-intensity, task-specific training of the limbs.[5] Robot-assisted gait training (RAGT) has been incorporated in many rehabilitation programs.[7] Many gait robots have been developed for use with treadmills, and RAGT has been shown to be effective in improving gait independence and gait speed in the acute setting.[8,9] However, for patients in the chronic phase with some walking independence, RAGT has provided less benefit. This is because the robots used in previous studies were designed to increase the level of gait training for non-ambulatory patients,[10] and they were not effective in changing the quality of gait for those who were already ambulatory and independent. However, reports in recent years have shown that robotic gait rehabilitation can reduce knee hyperextension and improve gait patterns for patients in the chronic phase in which the knee and ankle joints are controlled simultaneously and a near-normal gait pattern is repeatedly performed on a treadmill with assistance. Therefore, the possibility of improving gait in the chronic phase is emerging.[11]

It is known that RAGT affects knee kinematics, especially during gait, but has little effect on ankle motion.[12] This may be caused by the high prevalence of ankle joint paralysis in patients with CNS injury. Therefore, if training to induce intentional ankle joint movement can be performed in parallel with RAGT, the effects of RAGT may be extended to the ankle joint. Several single-joint robots that are attached to the ankle and trained in a seated position have been developed in recent years, and systematic reviews have shown that they can improve the ankle joint dorsiflexion angle during the swing phase and forward propulsion on the paretic side.[13] The Hybrid Assistive Limb Single Joint for Medical Use (HAL-MS01, Cyberdyne, Tsukuba, Japan) is a robot that practices ankle plantarflexion and dorsiflexion. It has been shown to increase muscle strength and improve the dorsiflexion angle of the ankle joint during the swing phase of walking after use in patients with peroneal nerve palsy and pediatric cerebral palsy.[14,15]

Given that the gait patterns of patients with central motor paralysis are created by deviation caused by motor paralysis and adaptation with compensatory movements for motor impairment,[16] we considered that it would be important to simultaneously approach motor paralysis and break free from deviant gait patterns. Therefore, we hypothesized that the gait pattern can be changed in chronic stroke patients by combining “RAGT to learn the gait pattern” and “ankle robot training to improve motor function”. In addressing this hypothesis, we report a case of intensive and comprehensive training using two robots to achieve improved motor function and gait pattern.

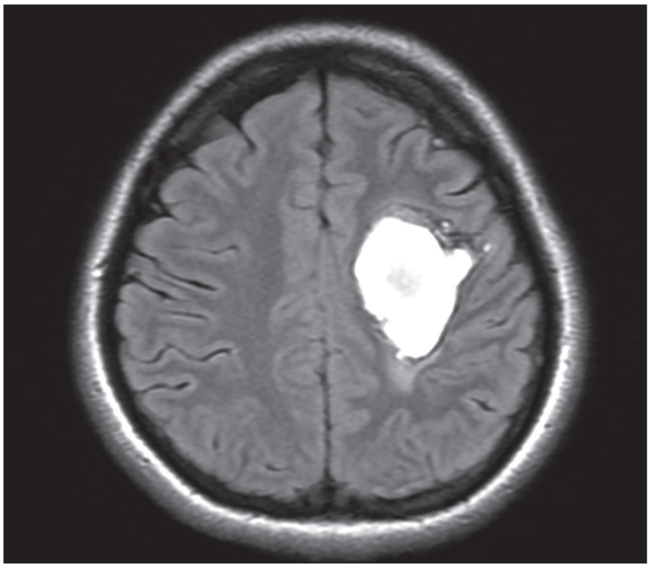

Fig. 1. Fluid-attenuated inversion recovery magnetic resonance imaging of patient at onset.

CASE

Patient Information

A 34-year-old woman developed a left frontal subcortical hemorrhage after trauma in 20XX (**Fig. 1**) and underwent convalescent rehabilitation. Although suffering right hemiplegia, the patient was able to independently perform indoor activities of daily living without prosthetic devices and could walk independently outdoors with a T-cane and a plastic short leg orthosis. She was admitted to hospital for rehabilitation therapy in 20XX+5. Treatment with botulinum toxin was administered approximately once every 6 months; the last administration was 54 days before the date of hospital admission. This study followed the guidelines of the Declaration of Helsinki and was approved by the Keio University School of Medicine Ethics Review Subcommittee (No. 20190246 and No. 20211008). Written informed consent was obtained from the patient for publication of the details of her case.

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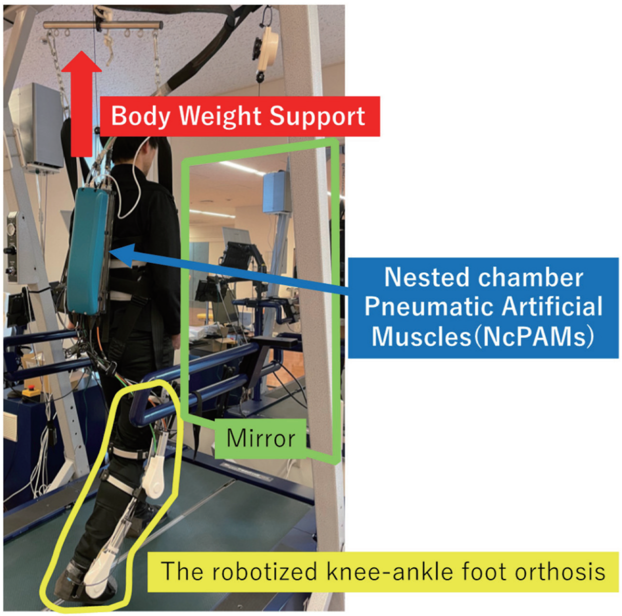

Fig. 2. Robotized knee–ankle–foot orthosis.

Robotized Knee–ankle–foot Orthosis for Learning the Gait Pattern

Figure 2 illustrates the exoskeleton robot device. This robotic device consisted of four parts: the exoskeleton body, which consisted of a metal support cuff, pneumatic artificial muscle (PAM) actuators, an operation computer, and a control computer.[11,17] The actuators used four nested chamber PAMs (NcPAMs) attached to two exoskeleton module joints at the knee and ankle joints. The NcPAM body was attached to the patient's back while suspended from the device, and the patient-borne robot weight was 2.9 kg. The two NcPAMs connected to the knee joint functioned in knee extension and flexion, whereas the two NcPAMs connected to the ankle joint functioned in plantarflexion and dorsiflexion. Parameter control devices were used to adjust the assist force and timing of each NcPAM. Assisted timing adjustments were applied as feedforward adjustments based on gait phase identification. Gait phase was identified by an algorithm based on foot pressure data obtained from foot force-sensing register (FSR) sensors; the FSR sensors were placed on the ball of the foot and the heel of the foot. FSR values during self-propelled walking were used to identify the gait phase of the patient. A life-size mirror was placed in front of the treadmill to provide visual feedback. The robot was designed to assist the knee and ankle joints in learning new gait patterns by repeating a near-normal gait pattern, thereby breaking out of the acquired deviant gait patterns (back knee, stiff knee, and drop foot).

In this study, RAGT was performed for 30 min per day, 2 or 3 days per week, for a total of 10 days. The training was performed with about 20% of body weight supported by an unloading device. During the training, the patient was given instruction to adapt to the robot's assisted movements. To ensure the safety of the training program, we developed and used a safety evaluation checklist.[11)]

Electromyogram-triggered Ankle Robot Training

The medical single-joint HAL® (HAL-SJ, HAL-MS01) is a wearable robot that can support flexion and extension movements of various joints, including elbow and knee joints.[14,18] In this study, the ankle joint attachment was used. It was attached to the outer side of the ankle joint and fitted with an actuator (motor) that triggered muscle action potentials from gel electrodes attached to the tibialis anterior and lateral head of the gastrocnemius muscles. This configuration was designed to assist in plantarflexion and dorsiflexion movements in accordance with voluntary movements,[14] and the level of assistance was adjusted with the controller. The controller was also equipped with a monitor to display electromyograms of the flexor and extensor muscles, thereby allowing the level of muscle activity to be monitored. Past reports of the use of the medical single-joint HAL® in patients with peroneal nerve palsy and cerebral palsy have described increased muscle strength, increased gait speed, and improvement in the maximum dorsiflexion angle during the free leg phase.[14,15] In the present case, voluntary movements were triggered, the assistance level was adjusted to allow the patient to perform bottom and dorsiflexion movements in the full range of motion, and 500 repetitions of bottom and dorsiflexion movements in the sitting position with knee flexion at 90° were performed daily. Assistance was provided in the dorsiflexion direction (red arrow in **Fig. 3**) when muscle activity from the tibialis anterior was high. When muscle activity from the gastrocnemius muscle was high, assistance was provided in the direction of plantarflexion (green arrow in **Fig. 3**). Visual feedback was provided by a lamp embedded in the actuator that turned red during dorsiflexion and green during plantarflexion.

Conventional Physical Therapy

Physical therapy for muscle strengthening training and joint range-of-motion training was provided for 20 min/day for 5 days per week. As self-training, the patient was instructed to do 100 half squats without full knee extension and roll a ball under the foot with flexion of the knee joint for 500 repetitions of voluntary exercise of the hamstrings. The

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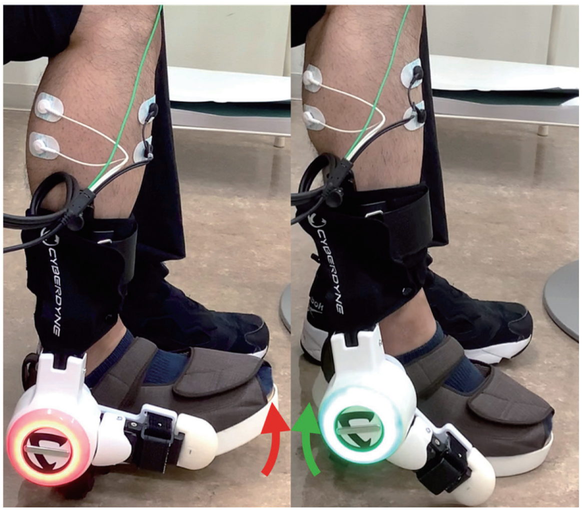

Fig. 3. Hybrid Assistive Limb Single Joint (ankle joint attachment). Red arrow indicates assistance provided in dorsiflexion direction. Green arrow indicates assistance provided in plantarflexion direction.

patient confirmed that these exercises were performed.

Assessments

Kinematic and Kinetic Data during Overground Gait

A three-dimensional (3D) motion capture system (Vicon, Vicon Motion Systems, Oxford, UK) was used for gait analysis. The reflective marker sets were chosen according to the plug-in gait lower-body model. Sixteen markers were attached to anatomical landmarks on both sides as follows: anterior superior iliac spine, posterior superior iliac spine, thigh, knee, tibia, ankle, toe, and heel. Motion data were sampled at a frequency of 100 Hz. A total of three gait cycles were measured. The examination was performed with the patient barefoot and without the use of an assistive device. Two force plates (MG1120, Anima, Tokyo, Japan) installed on the left and right sides recorded the ground reaction force (GRF) during walking at a comfortable speed without shoes over three gait cycles (sampling rate 100 Hz).

Kinematic and kinetic data were analyzed using MATLAB software (MathWorks, Natick, MA, USA). Both sets of data were time-normalized for each gait cycle. The mean value of the joint angle of the total gait cycle was calculated for each time point (0%–100%, total 101 points) and plotted on the graph. The trailing limb angle (TLA) is considered an important index of forward propulsion in analysis of gait following stroke.[19] The peak TLA angle was defined as the maximum sagittal angle between the vertical axis and the vector connecting the external ankle and greater trochanter of the paralyzed limb. The ankle dorsiflexion peak was defined as the maximum ankle dorsiflexion angle in the free leg phase. The peak knee joint flexion angle was defined as the maximum flexion angle of the knee joint during the insensate swing phase. The peak ankle joint plantar flexion angle acceleration was defined as the maximum ankle joint plantar flexion angle acceleration during the swing phase; there is a strong correlation between the plantar flexion angle acceleration at push-off and forward propulsive force.[20] The swing phase time ratio is a measure of the left–right temporal symmetry of gait,[21] with a value of 1 being symmetrical. As the ratio becomes smaller, the swing phase time on the paretic side becomes shorter and the gait becomes more asymmetrical. Data are expressed as mean ± standard deviation values.

GRF data were raw data smoothed using a 10-Hz Butterworth filter and normalized by patient weight (%BW).[22] Paretic propulsion is the integral of forward propulsion on the paretic side/non-paretic side of the anterior–posterior GRF, with 0.5 (50%) indicating perfect contrariness. Paretic propulsion has recently been used as an indicator of walking function in stroke patients.[23]

Clinical Assessments

Clinical assessments included the Stroke Impairment Assessment Set (SIAS) sensory score,[24] Fugl-Meyer Assessment-Lower Extremity (FMA-LE),[25] modified Ashworth scale (MAS),[26] and ankle clonus.[27] Gait ability assessments included the 10-m walking test (TMWT),[28] the 6-min walk test (6MWT),[29] and the Gait Assessment and Intervention Tool (G.A.I.T.).[30]

The SIAS has been used to assess hemiplegia in stroke and has been validated for internal consistency and predictive validity.[24] In this study, the SIAS sensory score was used to evaluate the tactile sensation of the dorsal foot and articulation of the great toe on a 4-point scale from 0 to 3, with higher values indicating normal function.

The FMA is widely used to evaluate motor function.[25] The FMA-LE is performed to assess movement, reflexes, speed, and coordination. The maximum score is 34, with higher scores indicating better function.

The MAS is a means of assessing spasticity and has been shown to be reliable and valid.[31,32] The knee flexors, knee extensors, and ankle plantarflexors were assessed before and after the intervention according to the assessment manual from a previous study.[33] Ankle clonus was measured manually by applying resistance in the dorsiflexion direction.[27]

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Table 1. Results of clinical assessments at pre- and post-training

Assessment	Pre-training	Post-training
Stroke Impairment Assessment Set sensory light touch/position test	2/2	2/2
Fugl Meyer Assessment-Lower Extremity (score)	18	23
I: Reflex activity	4	4
II: Synergistic movement	13	14
III: Mixed flexor and extensor synergy	1	3
IV: Isolated movement	0	0
V: Normal reflex activity	0	0
VI: Coordination/speed	0	2
Modified Ashworth Scale (knee extensor/knee flexor/ankle dorsiflexor)	0/0/1+	0/0/1
10-m walk test		
Comfortable gait speed (m/s)	0.57	0.69
Maximum gait speed (m/s)	0.64	0.78
6-min walk test (m)	240	288
G.A.I.T. (score)	35	27
Paretic propulsion (%)	5.59	4.99

The TMWT is commonly used to assess walking ability.[28] It measures speed during a 10-m walk. Currently, there are no clear rules regarding the acceleration and deceleration intervals before and after the 10-m segment. In the present study, a 3-m acceleration interval and a 3-m deceleration interval were set before and after the 10-m segment, and the 10-m walking speeds (comfortable and maximum) were calculated from times that were recorded with a digital stopwatch.[34] Two measurements were taken, and the faster of the two values was used. Evaluations were performed before and after the intervention.

The 6MWT was conducted according to the guidelines.[29] Because the test involves long-distance walking, it was performed with a T-cane and orthosis as used during normal long-distance walking.

G.A.I.T. is used for the evaluation of gait, with a score of 0 being considered normal, and a small score indicating that the gait is close to normal. The maximum score is 62.[30] In this study, the evaluation was performed at a comfortable walking pace without any assistive device or orthosis.

DISCUSSION

Table 1 shows the results of the functional assessments before and after the intervention. Physical function was assessed on the day of admission (Day 1): FMA-LE 18 points; MAS scores were knee extensor 0, knee flexor 0, ankle plantarflexor 1+; ankle clonus 20 beats; 10-m walk test speed 0.57 m/s comfortable and 0.64 m/s maximum without prosthetic devices; 6MWT 240 m; and G.A.I.T. 35 points. The 3D motion analyzer calculated the ankle dorsiflexion angle to be 3.22°, the knee flexion angle was 1.75°, and the swing time ratio was 1.67. GRF results showed that paretic propulsion was 5.59%.

RAGT was performed 2 or 3 days per week with Day 5 as the first day. Based on observational gait analysis, problem areas were identified as knee hyperextension, the decrease in knee joint flexion angle during pre-swing (PSw), ankle joint plantarflexion during push-off, and ankle dorsiflexion angle during the swing phase. Therefore, assistance was provided to knee flexion at mid stance (MSt) and PSw, ankle plantarflexion at PSw, and ankle dorsiflexion and knee extension from mid swing (MSw) to terminal swing (TSw). The final intervention of RAGT was completed on Day 27.

The post-intervention evaluation was performed on Day 28. The FMA-LE score was 23 points, and improvement in motor function was observed. The subitems were: IIA, foot dorsiflexion 1 to 2; III, knee flexion 1 to 2 and end-sitting foot dorsiflexion 0 (because of heel floating) to 1; and VI, coordination of tremor 0 to 1, and dysmetria 0 to 1. For spasticity, the MAS score for the ankle plantarflexors changed from 1+ to 1, and ankle clonus was 3 beats. Sensory scores were unchanged. Walking speed improved to 0.69 m/s (comfortable) and 0.78 m/s (maximum), and the G.A.I.T. score was 27. The distance for the 6MWT was 288 m. G.A.I.T. subitems were also changed: upper extremity flexion changed from 1 to 0; stance phase: weight shift changed from 2 to 1, knee hyperextension at MSt changed from 2 to 1, knee flexion at

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Table 2. Results of gait assessments in the experiment

Assessment	Pre-training	Post-training
Kinematic parameters		
Maximum knee joint flexion (degrees)	1.75 ± 1.98	16.54 ± 3.72
Maximum knee joint extension (degrees)	−18.72 ± 0.49	−17.74 ± 0.19
Maximum ankle joint dorsiflexion (degrees)	3.22 ± 3.42	12.59 ± 1.17
Maximum knee joint plantarflexion (degrees)	−13.31 ± 4.61	−7.49 ± 4.56
Maximum ankle joint plantarflexion velocity (degrees/s)	−139.33 ± 33.35	−145.79 ± 18.60
Trailing limb angle (degrees)	16.74 ± 0.83	17.44 ± 0.11
Temporal parameters		
Paretic side swing time (s)	0.65 ± 0.02	0.62 ± 0.06
Non-paretic side swing time (s)	0.40 ± 0.02	0.53 ± 0.01
Paretic side stance time (s)	0.77 ± 0.06	0.77 ± 0.11
Non-paretic side stance time (s)	1.03 ± 0.03	0.94 ± 0.02
Swing time ratio (paretic/non-paretic)	1.63 ± 0.12	1.17 ± 0.20
Stance time ratio (paretic/non-paretic)	0.75 ± 0.05	0.82 ± 0.09

Data are expressed as mean ± standard deviation.

terminal stance to PSw changed from 3 to 1, foot internal rotation changed from 2 to 1; swing phase: knee flexion at initial swing changed from 2 to 1, and knee flexion at MSw changed from 3 to 2. The results of kinematic evaluation using 3D motion analysis are shown in **Table 2** and **Fig. 4.** The swing time ratio had improved to 1.17. The results of GRF analysis for before and after training are shown in **Fig. 5.**

This is one of the few studies to have examined and reported the effects of combining two robots on motor function and gait pattern. Based on observational gait analysis, problem areas for the patient were identified as knee hyperextension, decreased knee joint flexion angle at PSw, ankle joint plantarflexion during push-off, and ankle joint dorsiflexion angle during the swing phase. To resolve these issues, the patient needed to gain ankle joint function and knee joint control. This required training to improve voluntariness and learning to control the knee and ankle joints during gait. When it became evident that one robot could not fulfill this task, a training regimen that used two robots was selected.

The first major effect was an improvement in the gait pattern. The minimal clinically important difference (MCID) in G.A.I.T. score in a chronic stroke patient with independent walking was shown to be 5.19,[35)] so the observed improvement of 8 points in the current case was deemed significant. The maximum ankle dorsiflexion angle during the swing phase improved from 3.22° to 12.59°. We believe that this was the result of learning voluntary movements through the assistance of the ankle robot by repeating dorsiflexion movements during walking in the RAGT and applying them to walking on level ground. The dorsiflexion angle during the swing phase is reported to be effective if it improves by 5°.[36)] This may also be influenced by a reduction in ankle joint spasticity, and co-contraction has been reported to have decreased with the use of the HAL®.[15)] However, no improvement in plantarflexion was obtained in the present study. Therefore, the floor reaction force data did not show any improvement in paretic propulsion, and no improvement in push-off was observed in terms of kinematics. However, there was a decrease in force in the direction that inhibited the forward propulsion at the end of the stance phase (blue arrow in **Fig. 5**). This may suggest the effect of learning lower leg forward tilt because of knee joint flexion at the end of the stance phase.

The knee flexion from the stance cycle to the middle of the swing phase also showed improvement, and similar results were obtained in the 3D motion analysis shown in **Fig. 3.** The MCID of the knee joint flexion angle is reported to be 8.48°,[37)] which suggests significant improvement in this case. This result is consistent with a previous study that used the same robot [11)] We believe that knee flexion pattern learning during walking was achieved by using the lower limb robot. Temporal symmetry was also improved. Symmetry is reported to worsen in stroke gait.[38)] Given that gait asymmetry may be associated with a number of negative outcomes, including inefficiency, balance control challenges, risk of musculoskeletal damage in the non-paralyzed lower extremity, and reduced bone density in the paralyzed lower extremity,[39)] its improvement is of clinical importance.

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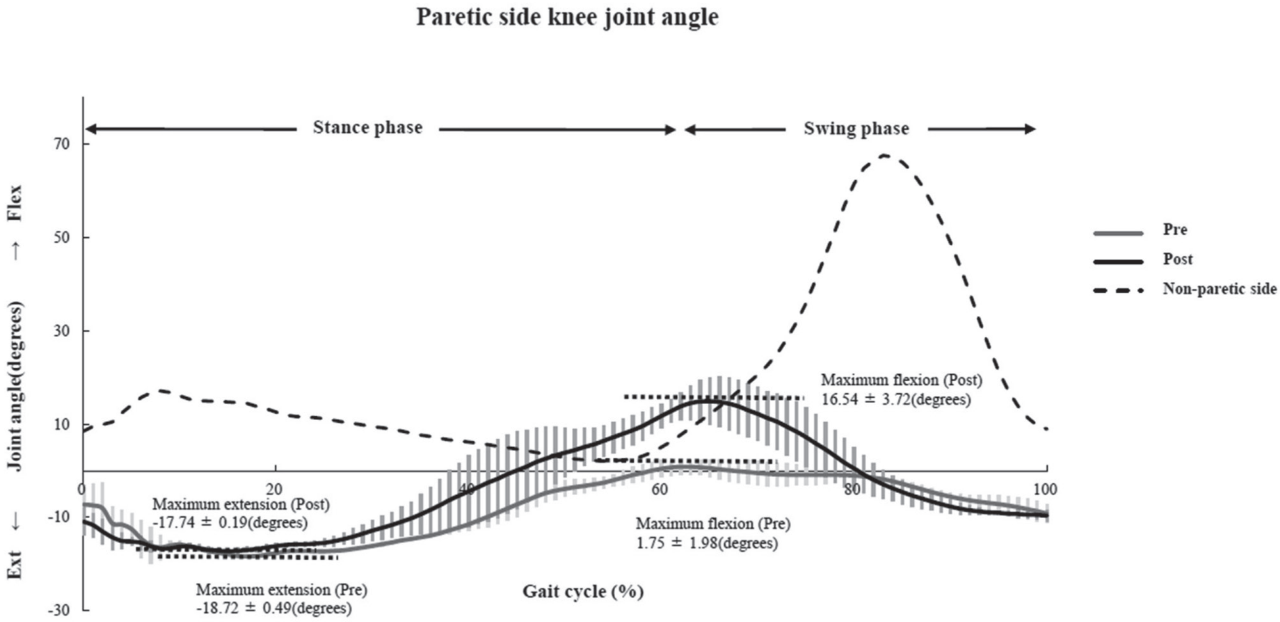

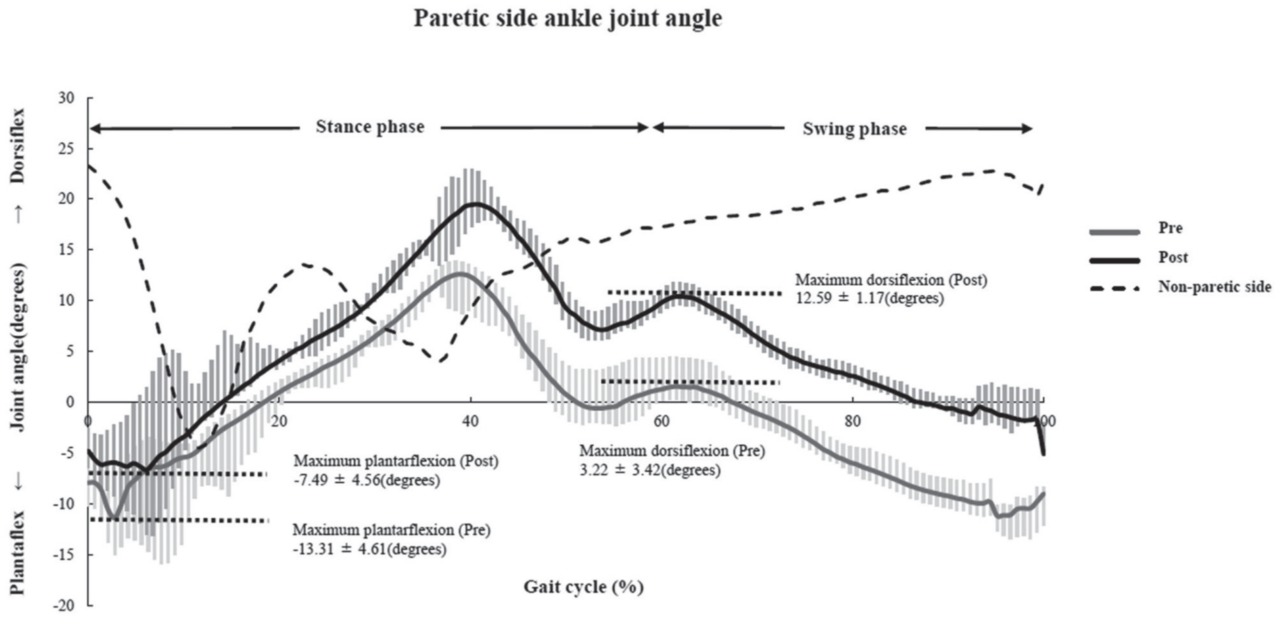

Fig. 4. Changes in knee (upper) and ankle (lower) joint angles before and after intervention during comfortable gait.

Improvements were noted for motor function of the ankle and knee joints. Apart from one randomized, controlled trial that reported a 5.7-point improvement in FMA-LE in the chronic phase by combining the Lokomat (Hocoma; Zurich, Switzerland) and conventional physical therapy,[40] no other robot has shown any effect on motor function improvement, and evidence for motor function improvement by RAGT is still lacking. The robotized knee–ankle orthosis used in the present study was especially focused on gait pattern improvement in the intervention, and it is considered that the improvement of motor function of knee flexion (from 1 to 2 in the FMA-LE) was obtained by learning the gait pattern motion of knee flexion during walking, and similar improvement of the motor paralysis of flexion pattern was also obtained in a study using the same robot.[11] It is highly unlikely that use of the existing walking robot alone will improve motor paralysis of the ankle joint, but there are reports that use of the HAL® in the sitting position increases

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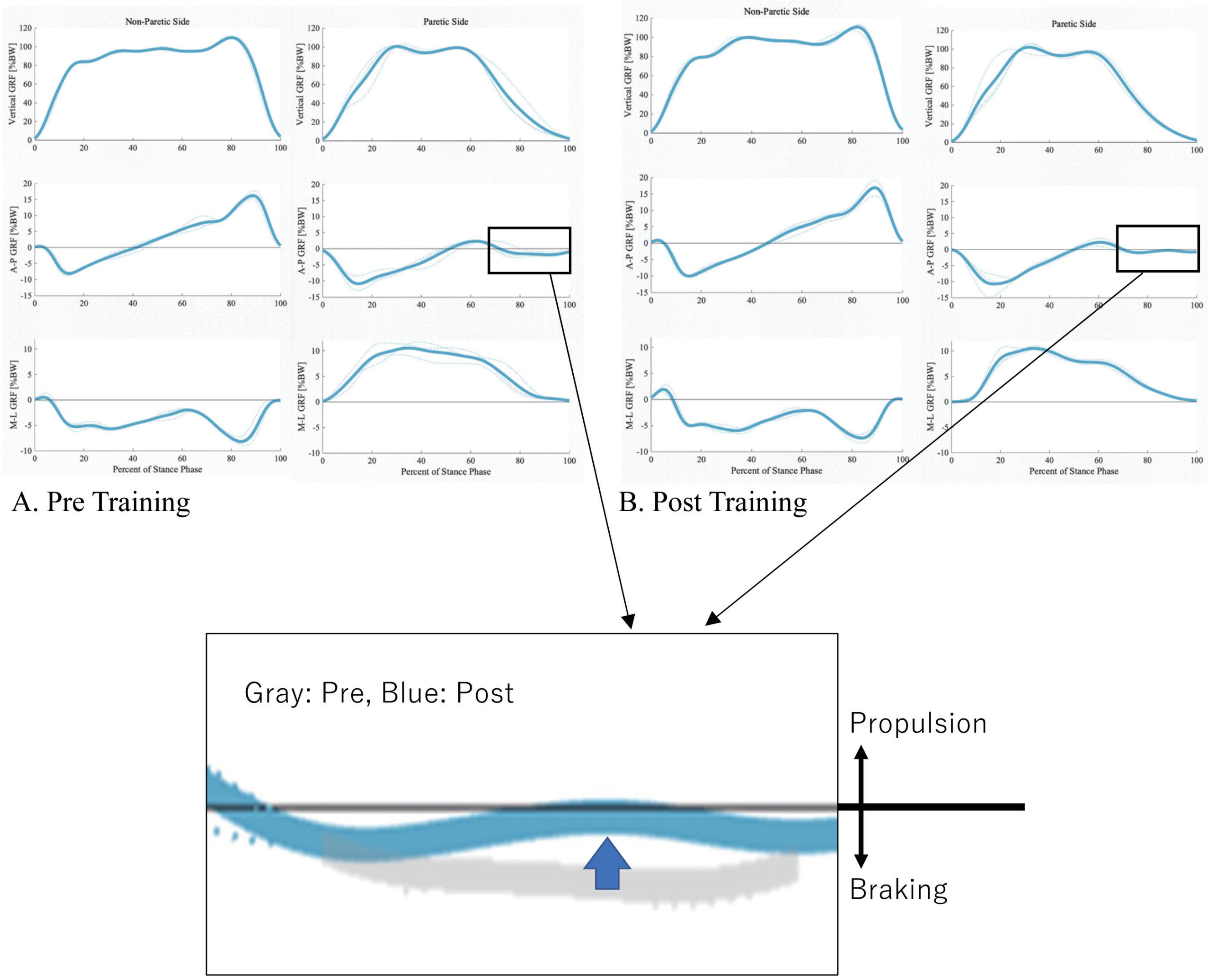

Fig. 5. Ground reaction force (GRF) during comfortable gait at pre-training (A) and post-training (B). The blue arrow shows that the force in the direction that inhibits forward propulsion in the terminal stance is reduced. BW, body weight; A-P, anterior–posterior; M-L, medio-lateral.

muscle strength and increases the dorsiflexion angle of the ankle joint during the free leg phase of walking after use in peroneal nerve palsy and childhood cerebral palsy.[14,15] The results of the present study are consistent with these reports. Based on the above, we believe that the combined use of two robots, a walking robot and an ankle robot, may have improved motor function in the knee and ankle, respectively.

Given that the reported MCID for the 6MWT is 44 m,[41] the improvement of 48 m observed in the current study suggested that walking endurance was significantly improved. In general, body-weight-supported treadmill training (BWSTT)-RAGT is associated with greater improvements in dynamic balance, speed, and endurance during walking than the use of BWSTT alone.[42] We believe that use of the robot may have allowed the patient to learn to walk more efficiently, resulting in improved gait efficiency. As one of the strategies, the increased swing time on the non-paretic side and the increased braking force seen in **Fig. 5** suggest that the non-paretic side may have widened its stride length. However, with regard to walking speed, the minimal detected change in a chronic stroke patient with medium speed (0.4–0.8 m/s) was 0.15 m/s,[43] whereas the present case improved by 0.12 m/s, which was not significant. This result may have been influenced by the treadmill speed during RAGT, which was a maximum of 2.0 km/h (0.56 m/s), because this RAGT prioritized a gait approach. The walking speed protocol during

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RAGT should be investigated in the future.

It is known that improvement of hyperextension of the knee is difficult in patients with chronic conditions. Systematic reviews have shown that treatment for knee hyperextension includes proprioceptive training that maintains knee flexion during gait and exercise.[6] In the present case, knee flexion during the stance phase of walking with RAGT and half squats without full knee extension during self-training did not improve knee hyperextension. However, spasticity of the ankle joint, one of the causes of knee hyperextension, showed a tendency to improve with this intervention (MAS of ankle dorsiflexion changed from 1+ to 1). Continued repetitive gait training with controlled ankle spasticity and knee hyperextension was considered necessary.

This study had some limitations caused by the prior use of botulinum toxin. Considering that the effect of botulinum toxin peaks after about 2 weeks and gradually decays from there,[44] it is unlikely that it affected the patient because 54 days had passed from the time of injection to the time of admission. However, an effect could not be completely ruled out. In addition, it is difficult to compare data from before botulinum toxin treatment and at the time of admission because it is not possible to present data on motor function and walking function at the time before botulinum toxin was administered.

CONCLUSION

This study presented a case in which the combination of ankle robot training and RAGT improved motor function and the gait pattern. Examination of further cases is needed to confirm whether treatments using a combination of robots may lead to improvements of motor function and gait, even in the chronic phase.

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CONFLICTS OF INTEREST

The Department of Rehabilitation Medicine (Keio University School of Medicine) has a joint research agreement with Cyberdyne Inc. Although this study benefitted from the free loan of the ankle robot (HAL®), Cyberdyne was not directly involved in the study design; collection, analysis, or interpretation of data; preparation of the manuscript; or the decision to submit it for publication. The authors declare no conflict of interest.

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