Clinical trial to compare conventional multidisciplinary gait rehabilitation with the Atalante self-balancing walking system in patients with Multiple Sclerosis

Protocol

Modification Date	Modification Type
09.11.2022	Draft version 1
16.11.2022	Comments from IG applied
18.11.2022	Comments from GL, CS applied
18.11.2022	Comments from CS applied
31.01.2023	Version 2 approved by the Ethics Committee
16.01.2024	Amendment: Redefinition of outcomes and inclusion of epigenetic outcomes. Validation and approval by the Ethics Committee. **Final version in Spanish without annexes**

Index

1 Justification

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system (CNS), affecting more than 2 million people worldwide1. It is a chronic and degenerative disease that selectively affects the CNS and represents the leading cause of non-traumatic disability in young adults. The age of onset of the disease is usually between 20 and 40 years. The prevalence of MS in Spain fluctuates between 40 and 125 cases per 100,000 inhabitants, with an incidence of 3-4 new cases per 100,000 inhabitants and year. 2

Clinically, the disease initially presents in more than 80% of cases in a relapsing-remitting form, that is, in the form of outbreaks or exacerbations in one or several locations with periods apparently stable between two of these episodes. After a variable period of time, a progressive deterioration appears usually associated with important accumulation of disability. The symptoms of the people with MS (PwMS) are very variable and differ from one patient to another in topography and intensity. Thus, we can find visual, sensory symptoms, loss of strength, sphincter and sexual alterations, coordination problems, balance problems, cognitive alterations and fatigue among others. [3]

Gait and balance disturbances in MS are already common even in the early stages of the disease. Half of the patients refer some alteration in the quality of their gait within the first month after diagnosis, reaching 90% at 10 years of evolution.[4] [5] Furthermore, gait is the most valuable body function for MS patients, both for those with less than 5 years of evolution and for those with more than 15 years, as walking restriction hardly affects their activity and participation level. [7]
Gait disturbance is caused by multiple factors such as decreased muscle strength, balance, coordination, and proprioception, impaired vision, spasticity, fatigue and even cognitive impairment.[4]
Some impairments arise in the phisical and social context of each patient, limiting participation in activities or therapies to improve balance, fatigue, strength and gait characteristics such as fatigue, high risk of falls or high disability.[7]

This would support the development and application of rehabilitation strategies for mitigating the energetic penalties of gait abnormalities during ambulation. Some of these interventions include aerobic, resistance, yoga, and combined exercise training, that have shown significant improvements in walking endurance regardless of outcome measures (6MWT, 2MWT). [8]
New emerging approaches might involve Robot-assisted gait training In the last 5 years a lot of robotic solutions that offer both strategies of support in dialy living and rehabilitative devices have appeared. Some studies have shown that people with multiple sclerosis and severe gait impairment who participated in Robot-assisted gait training (RAGT) achieved significant improvement in lower limb muscle strength and increased their walking speed, although the effect was not long-lasting.[9]

In the last years evidence about robot-based rehabilitation for PwMS has been published, and currently, more than 5 devices have been approved for its use in gait neurorehabilitation. Yeh et al. concluded that Robotic locomotor training limitedly affects motor functions of multiple sclerosis, but improves fatigue and spasticity, it is safe and well-tolerated for PwMS and less demanding for physical therapists.[10] Bowman et al. concluded that Robotic-Assisted Gait therapy (RAGT) improves balance and gait out-

comes in a clinically meaningful way in PwMS, RAGT seems more effective compared to conventional rehabilitation, while it shows similar effects if compared to specific balance and gait training in studies with level 2 evidence.[11] RAGT has several advantages in terms of patient motor assistance, intensity of training, safety, and the possibility to combine other therapeutic approaches and should be promoted for PwMS with severe disability in a multimodal rehabilitation context as an opportunity to maximize recovery.[11] In this context is needed a further larger-scale, better-designed RCTs with a longer training duration and more studies evaluating the satisfaction, usability and effectivity of RAGT.[10 12 13 11]

The Atalante exoskeleton is composed of an external, powered, motorized orthosis that is placed over a person's limbs to provide self-ambulation functions without the use of crutches or other technical aids. The main feature of the exoskeleton is that it is fully actuated with 12 actuated degrees of freedom: three at each hip, one at each knee, and two at each ankle. [14 15]

The molecular bases associated with the functional improvements derived from rehabilitation therapies or physical exercise are not yet well defined. However, both rehabilitation and exercise have been recognized for their immunomodulatory properties. In fact, recent clinical and preclinical findings have established a connection between physical activity and rehabilitative interventions with discernible epigenetic alterations in immune cells. For example, recent preclinical research has demonstrated how moderate-intensity training induces lasting changes in chromatin accessibility in macrophages, resulting in moderation of their inflammatory responses. However, there is still a lack of comprehensive epigenomic studies examining the repercussions of rehabilitation therapies in individuals with multiple sclerosis.

2 Aims

2.1 Primary Aim:

- To compare the effect of RAGT by use of Atalante self-balance exoskeleton with conventional rehabilitation training (cRHB) for improving **Walking speed** measured by the Timed 10-meters walk test (10-MWT), considering a clinically relevant change an improvement of 20% of the velocity (m/s) with respect to the baseline assessment.[16]

2.2 Secondary Aims:

- To compare the effect of RAGT by use of the Atalante self-balance exoskeleton with conventional Rehabilitation training (cRHB), for improving **static balance, dynamic balance,** mobility, and **self-perception of gait impairment** measured with:

- **Static Balance** by the Berg Balance Scale (BBS), considering a clinically relevant change for improvement in balance as measured by BBS was +3 points, meaning that PwMS are likely to perceive that as a reproducible and clinically important change in them balance performance.[17]
- **Dynamic balance** by the Timed Up and Go test (TUG)[18]
- **Mobility** measured by modified Rivermead Mobility Index (mRMI)[19]

- **Self-perception of gait** impairment by Multiple Sclerosis Walking Scale-12 (MSWS-12)[20]
- **Instrumented gait analysis** by the G-walk device (assessing gait cycle duration, step length, propulsion index, support phase percentage D/I, oscillation phase percentage D/I, double/unique support phase percentage.

- To compare the effect of RAGT by use of the Atalante self-balance exoskeleton with conventional Rehabilitation training (cRHB) on quality of life, emotional status and fatigue measured at pre and post-intervention with:

- Multiple Sclerosis Quality of Life-54 (MSQoL 54 Health Survey Spanish version)[21]
- Hospital Anxiety and Depression Scale (HADs) considering a clinically relevant change of 1.7 points at HADs[22]
- The Modified fatigue scale (MFIS) [23] [24]

- The patient' physical effort of each session by means of the scale of perception of the effort R.P.E of Borg at the end of each training[25] [26].
- To evaluate the epigenetic blueprint of rehabilitation training by comparing the genome wide chromatin accessibility of peripheral blood mononuclear cells (PBMCs) before and after RAGT or cRHB, by Assay for Transposase-Accessible Chromatin sequencing (ATACseq).

- To test the hypothesis that genome wide chromatin accessibility compared to clinical measures can be useful for identifying molecular mechanisms underlying rehabilitation therapies.

- To compare the changes induced by RAGT versus cRHB on the overall chromatin accessibility, and specific genome regions and pathways.

- Use the biological samples to provide additional functional and mechanistic information about the rehabilitation effects and subject health, disease or state.

3 Materials And methods

3.1 Design:

This trial is a prospective, randomized, rater-blinded study (cRHB (N=30) vs RAGT (N=30)).
For the control group, this protocol includes 24 one-hour sessions of cRHB training during 8 weeks three times per week, under the supervision of a qualified rehab team.
For the intervention group (RAGT) this protocol includes 24 one-hour sessions of RAGT during 8 weeks three times per week, under the supervision of a qualified rehab team.
Additionally, peripheral blood (6ml) will be taken from all subjects at three timepoints: at baseline (T1), after the 8 weeks of rehabilitation treatment (T2) and, if possible, one year after T4. Biological samples will then be given a unique identifier and transferred to researchers for analysis.

Informed consent will be obtained from patients prior to inclusion in the study, which will be carried out in accordance with the Declaration of Helsinki.

3.2 Inclusion and exclusion Criteria

Subjects will be evaluated for their eligibility from neurorehabilitation and neurology outpatient clinics at the Multiple Sclerosis Center of Catalonia (Cemcat).

3.2.1 Inclusion criteria

- Male or female, between 18 and 75 years of age.
- Confirmed diagnosis of MS.
- EDSS from 6.0 to 7.0.
- Able to maintain the upright position on a daily basis.
- Stable course of disease-modifying therapy over the past 6 months.
- Clinical comorbidity asymptomatic (i.e., no underlying cardiovascular disease)
- Height: between approximately 1.50 m. and 1.90 m.
- Willingness to visit the Multiple Sclerosis Center of Catalonia (Cemcat) for testing and training.
- Gait disorder conditioned by paresis or hemiparesis associated with ataxia or sensory problems.
- Patient having given written consent.
  Atalante is able to accommodate the following limb lengths:
- Thigh: 380-460mm.
- Distance between the ground and the joint space of the knee (to be measured while wearing the shoes they intend to wear with Atalante):
  - 457–607mm for patient with an ankle dorsiflexion ≥ 16°
  - 457–577mm for patient with an ankle dorsiflexion between 13° et 16°
  - 457–567mm for patient with an ankle dorsiflexion between 10° et 13°
  - 457–557mm for patient with an ankle dorsiflexion between 0° and 10°
- Hip with less or equal to 460mm when seated.
- Maximum weight: 90 kg.

3.2.2 Exclusion criteria

- Pregnancy.
- Starting or switching from fampridine (Fampyra®) in the last 4 weeks.
- Height and weight outside the secure standard of safe use, described in the safety guides.
- Contraindications for Atalante training (eg, bone instability, history of osteoporosis or osteoporotic fractures).
- Subjects under Corticosteroids treatment or relapse.
- Changes in disease-modifying and symptomatic therapy for MS during the study period.
- Subjects with psychiatric or cognitive comorbidities that may interfere with the trial.
- Whose joint centers cannot be aligned Atalante's.
- Ranges of motion below:
  - Knee: 5° extension, 110° flexion

    - Ankle: 0° dorsiflexion, 9° plantar flexion, 18° inversion and eversion
    - Hip: 115° flexion, 15° extension, 17° abduction, 10° adduction, 10°
    - medial rotation, 20° lateral rotation
- Severe spasticity (greater than Ashworth 3) or uncontrolled clonus.
- Severe concurrent medical diseases: infections, circulatory, heart or lung, pressure sores.
- Active implantable medical device.

3.3 Enrollment and Screening

Subjects will be evaluated for their eligibility from neurorehabilitation and neurology outpatient clinics at the Multiple Sclerosis Center of Catalonia (Cemcat).

Potential subjects will be referred to the study via Cemcat Physicians. A member of the research team will screen the referral and confirm they meet the necessary inclusion and exclusion criteria. Following confirmation of criteria, potential subjects will be contacted by phone to schedule an initial visit for consent and baseline assessment.
Additionally, peripheral blood (6ml) will also be taken from all subjects at baseline (T1).

Prior to all testing, each of the 60 selected participants will have time to read the consent form and ask questions (see Draft Informed Consent). The consent process will take place at the Multiple Sclerosis Centre of Catalonia (Cemcat, Vall d'Hebron Barcelona Hospital Campus, Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona).

3.4 Assessments

The following information will be collected for all patients: age, sex, weight, height, type of MS, years from the diagnosis, Expanded Disability Status Scale (EDSS) and type of support device.

To ensure that training sessions are well tolerated, we will assess patients' blood pressure and heart rate weekly. We will also monitor the appearance of pain in any part of the body related to the RAGT at end of each training session using a pain registry (type of pain, location, intensity)[27].

3.4.1.1 To reduce the incidence of any type of muscle injury the physical therapist before each RAGT session will assess the level of spasticity using the Numeric Rating Scale measure of spasticity (NRS). The 0–10 NRS asks patients to indicate their level of spasticity on a scale of 0–10, where 0 is no spasticity and 10 is the worst possible spasticity. This type of rating tool is widely utilized in clinical settings to quickly assess pain.[28]

A record of any unexpected Serious Adverse Events (SAE) within trial episodes will be keep. These are defined as death, a life-threatening adverse event or an event occurring as a result of the use of the device that requires medical intervention.

After the 8 weeks of rehabilitation treatment (T2) and, if possible, one year after T4, peripheral blood (6ml) will be taken from all subjects to perform the subsequent molecular analysis.

Molecular analysis

Peripheral blood mononuclear cells (PBMCs) will be purified using the Ficoll-Hypaque density gradient method according to manufacturer's instructions. Briefly, whole blood will be extracted by venepuncture and collected in CPT Vacutainer tubes (Becton Dickinson, BDAM362781) and centrifuged at 2250g for 25 min. Supernatant will then be collected, and washed twice with enriched saline (0.9% NaCl, 2%FBS). PMBC containing pellet will then be resuspended with 1 ml of complete media (RPMI1640 + 2% FBS) and cells will be counted and assessed for viability using a Countess 3 automated cell counter (Invitrogene). PBMCs will be adjusted to 5 × 106 cells/ml in freezing media media (90% FBS, 10% DMSO), and frozen at − 80 °C using a Mr. Frosty™ Freezing Container (Thermo Scientific™) for 24 h and stored in liquid nitrogen vapor phase.

Once all samples are collected, chromatin will be extracted from the frozen PBMCs. Briefly, each vial containing 5 × 106 cells will be thawed and centrifuged at 300g for 5 min at 4°C. The supernatant will then be removed, and cells will be mixed with 100µL of lysis buffer (10 mM NaCl, 3 mM MgCl2, 10 mM Tris–HCl pH7.4, 0.1% Tween-20, 0.1% NP40) and lysed on ice for 4 min. Followed by 2x1ml washes (10 mM NaCl, 3 mM MgCl2, 10 mM Tris–HCl pH7.4, 0.1% Tween20) before centrifuging at 500g for 5 min at 4°C.
Next, ATAC-seq libraries will be generated according to manufacturer guidelines. Briefly, transposition mix (Illumina, Cat# 20031198) will be added to the nuclear pellets, incubated at 37 °C for 30 min, and DNA purified using the QIAGEN MinElute PCR Purification Kit (QIAGEN, Cat#28004). DNA fragments will be amplified by PCR for 10 to 11 cycles and resulting libraries purified using the QIAGEN MinElute PCR Purification Kit. The libraries will then be sequenced with an Illumina Novaseq 6000 S4 flow cell using 150 bp paired-end reads (Illumina, Cat# 20027466). FASTQ files will be generated from the NovaSeq BCL outputs and used as input to the ENCODE ATAC-seq pipeline (https://github.com/ENCODE-DCC/atac-seq-pipeline) using the MACS2 peak-caller with all default parameters. Differential accessibility will be initially calculated between groups using the csaw package.
To identify differentially open chromatin regions between groups from ATAC-seq, the R package edgeR will be used to fit a generalized linear model (GLM) to test for the effect of rehabilitation between T0 and T1 samples. In addition to time group (T0 vs. T1), we will also compare the two different rehabilitation treatments (cRHB vs. RAGT at T1). We will also use surrogate variable analysis (SVA) to capture unknown sources of variation (e.g., batch effects, age, sex, library preparation, etc) statistically independent from time or treatment group assignments. SVA decomposes the variation that is not accounted for by known factors such as time group or treatment into orthogonal vectors that can then be used as additional covariates when fitting a model to test for differential accessibility or expression.
Benjamini-Hochberg P value correction will be used to select differentially open peaks at an FDR of 5%.
Differentially open ATAC-seq peaks will then be annotated with regard to functional and positional information. HOMER will be used to annotate peaks based on the relative gene position (promoter (±2 kb of known TSS), intergenic,

intronic, among others). For functional annotation of peaks, we will use a simplified version of the 18-state ChromHMM-derived chromatin states (such as TSSs, enhancers, repressed polycomb, transcriptionally active, or other states such as ZNFs or heterochromatin) obtained from Roadmap Epigenomics data for PBMC and T cell subsets.

For gene-based analyses, HOMER will be used used to assign each differentially open ATAC-seq peak to the nearest TSS, as measured from the peak center. We will then assess enrichment using the hypergeometric test followed by Benjamini-Hochberg FDR adjustment for P values, as previously mentioned.

Further functional and ontological enrichment analyses will be performed using ClueGO and WikiPathways that will allow us to identify pathways among genes associated to differentially open peaks.

Finally, we will scan the data for transcription factor (TF) footprints using the PIQ algorithm which integrates genome-wide TF motifs with chromatin accessibility estimates profiled at base pair resolution to generate a list of possible footprint matches for a motif. In a nutshell, this will allow the identification of chromatin accessibility blueprint generated by the different rehabilitation therapies tested in this trial, as well as gather valuable data of its underlying molecular mechanisms that in turn might lead to the identification of novel therapeutic targets.

Type outcome	Test		Baseline (T1)			RAG Session 1-24 (S1-S24)			end of training (T2)			Follow up at 2 months (T3)			Follow up at 1 year (T4)		
			CRO	PROMs	Blood sample	CRO	PROMs	Blood sample	CRO	PROMs	Blood sample	CRO	PROMs	Blood sample	CRO	PROMs	Blood sample
Pain	1	Pain registry		x			x			x			x				
Physiologic indicators	2	Heart rate	x			x			x			x					
	3	Arterial pressure	x			x			x			x					
Effort	4	Rating perception exertion The Borg R.P.E scale					x										
Fatigue:	5	Modified fatigue scale (MFIS)		x						x							
Depression	6	Hospital Anxiety and Depression Scale (HADs)		x						x							
Health-related quality of life	7	MSQoL 54 Health Survey		x						x							
Mobility	8	Modified Rivermead Mobility Index	x						x			x					
Disability	9	Expanded Disability Status Scale (EDSS) de Kurtzke	x						x			x					
Balance	10	Berg scale test and Timed Up and Go test	x						x			x					
Gait	11	Timed 10-meters walk test	x						x			x					
	12	self-report 12-Item Multiple Sclerosis Walking Scale		x						x							
	13	G-walk Assess (gait cycle duration, step length, propulsion	x						x			x					

		index, support phase percentage D/I, oscillation phase percentage D/I, double support phase percentage, unique support Phase Percentage															
Spasticity	14	NRS		x			x			x		x					
User Satisfaction	15	Gagnon scale		x			x										
Factors epigenetics	16	Homer Gene Based analyses			x						x						x
IC, Informed consent; Patient-Reported Outcome Measures (PROMs), Clinic report, CRO;																	

Table 1Assessments

3.4.2 Schedule

Following completion of day 1 assessments and randomization, all subjects will be scheduled for the intervention phase of the study. Each participant will receive 24 sessions of RAGT or cRHB scheduled for three times a week for 8 weeks. If a subject cancels a treatment session, it will be counted as missed session. If a subject misses three consecutive treatment sessions, they will be dropped from the study due to inconsistency in treatment carryover. Cancellations and changes in patient scheduling represent a true clinical environment and represents normal clinical patient management.
At the end of the intervention phase, a new exploration will be carried out.

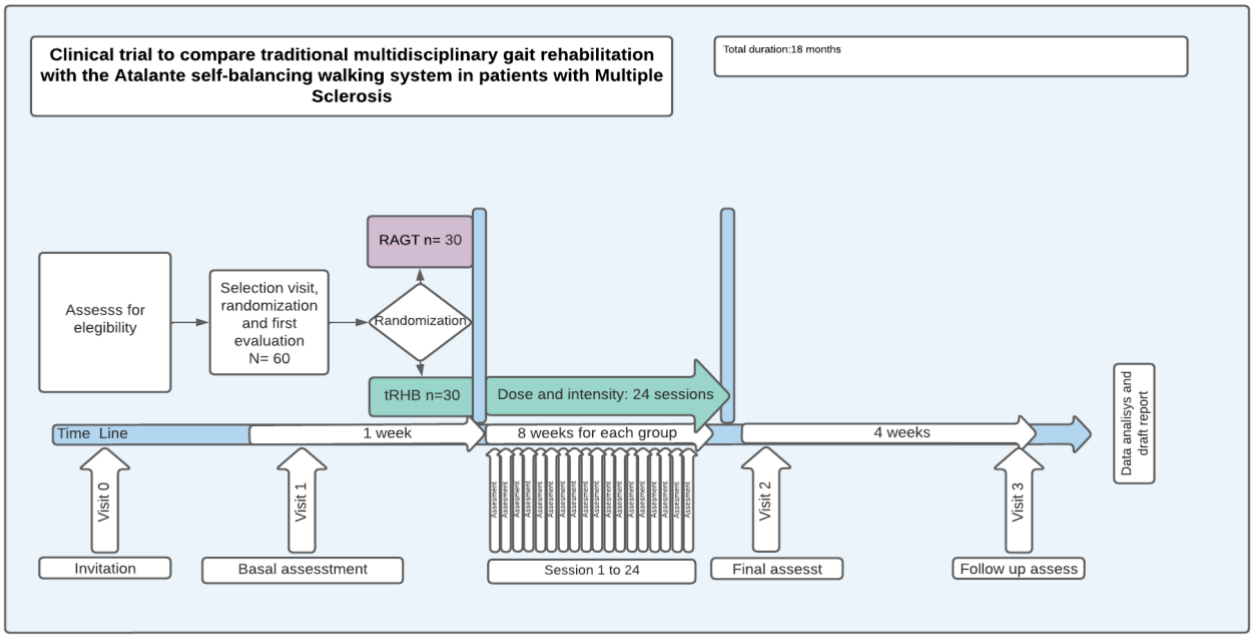

Figure 1 schedule

3.5 Device

3.5.1 Description

Atalante is the first exoskeleton designed and developed by Wandercraft. Atalante is intended to perform ambulatory functions, mobility exercises and adapted physical exercises, hands-free for individuals having lower limb and trunk disability in rehabilitation centers, city cabinets or adapted physical exercise centers under the supervision of a trained operator.

The device is available with one unique model and allows for length mechanical adjustment of specific parts so that patients with different physical characteristics can undergo care sessions with the same unit of Atalante - after it has been adjusted to their own set of measurements.

Figure 2 shows the main parts of the device.

Figure 2: Atalante main parts

Atalante is an exoskeleton composed of:

- **Two operated legs** made up of articulated parts: thigh, leg, and foot. These parts can move thanks to 12 actuators comprised each of an electric motor, a position sensor (encoder), and a motor controller board to process related data. Figure 3 shows the location of each of the actuators. In addition, the thighs and legs are adjustable in length to allow adaptation to different patient morphologies. The lower structure is made of aluminium alloy, parts of which are covered by plastic shells.
- **A back**, made of composite material, which links the lower limbs together and supports the upper body of the patient.
- **A vest**, made of fabric, and equipped with an inertial sensor (IMU) to detect the intention of the patient inside the exoskeleton based on the motion of his chest. The vest is available in two sizes: small (S) and large (L).
- **Five additional inertial sensors** located in the back, legs and feet for orientation estimation of different parts of the exoskeleton.
- **Force sensors** placed in the feet for contact state estimation.
- An **embedded computing system** (Wanderbrain and Wanderneuron software) to retrieve data from all sensors and boards, and coordinate motion - determining commands for the actuator blocks.
- A **set of plastic wedges** to adapt to the patient morphology. The wedges compensate for a less than 16° dorsal flexion of the ankle and/or increase the distance between the patient's knee and Atalante's foot. They exist in 8 different sizes in terms of inclination (between 0 and 16°) and height and they are placed on Atalante's feet, if needed.
- A **battery system** consisting of two pluggable and rechargeable packs of Lithium-Ion cells enclosed in plastic casings and located each on the thighs.

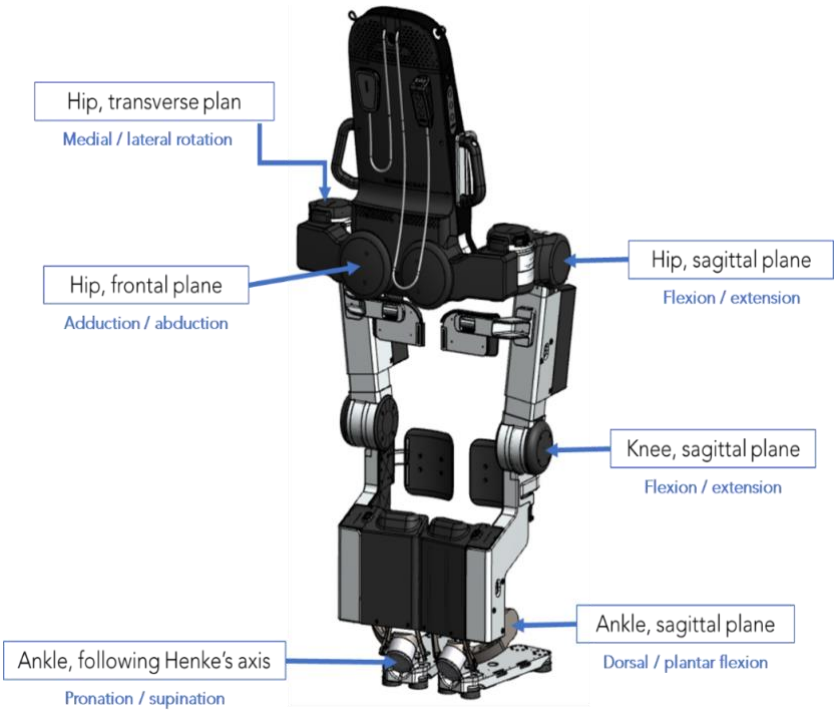

Figure 3: Atalante joints

The ranges of motion of the 12 joints are constrained by mechanical stops and supplemented by software stops to protect the patient's joints from exceeding motion limits. Atalante is attached to the patient in 3 locations per leg (thigh, leg, foot) plus at the abdomen level thanks to the vest's belt. These interfaces are made of rigid shells covered with foams and equipped with adjustable straps that maintain the patient and facilitate transmission of the system movements to the patient, all while ensuring their comfort. The rigid shells are located at the back of the patient's thigh and at the front of the leg.
Atalante is controlled (Figure 4) by its user thanks to:

- Sensors, especially a motion sensor (i.e., the IMU) placed on the back of the vest which triggers the movement.
- Two keyboards, one for the operator (physio controller) leading the care session and one remote intended for either the operator or optionally the patient.

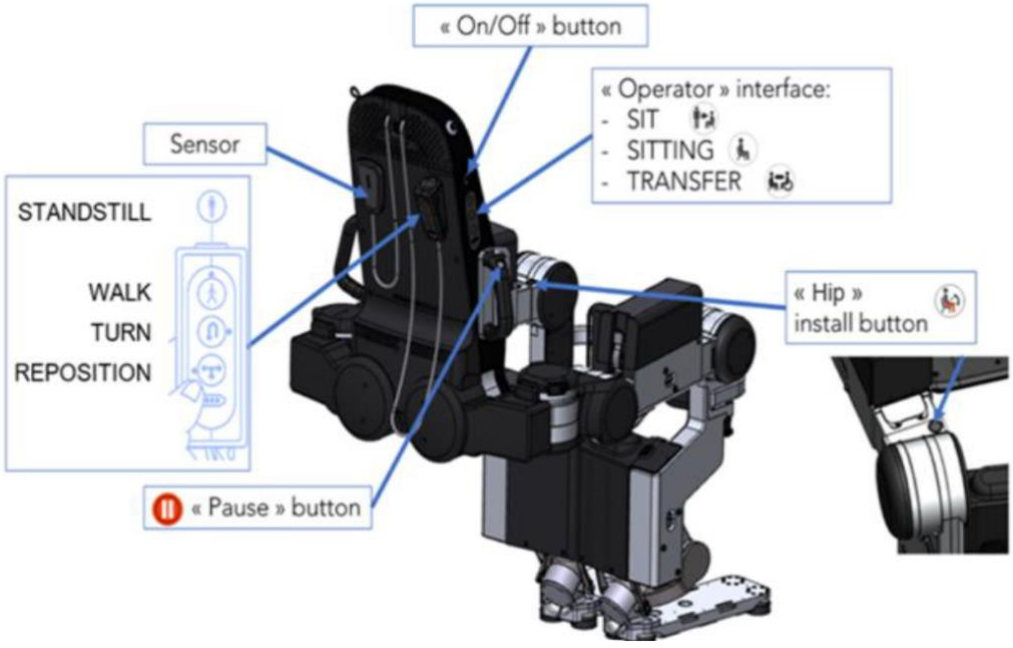

Figure 4: Control interface of Atalante

As Atalante is destined to be used in healthcare facilities, city cabinets or adapted physical exercise centers with several patients in a day, the angular amplitudes of each articulation have been designed to allow a broad range of patients to be included into the exoskeleton.
Furthermore, the device is equipped with mechanical adjustments allowing the length of the legs and the thighs to be regulated in the range 40-49 cm and 38-46 cm respectively, according to the dimensional measurements of the patients. The angular amplitudes of the 12 motorized articulations and the mechanical adjustment ranges are shown in the figure below.

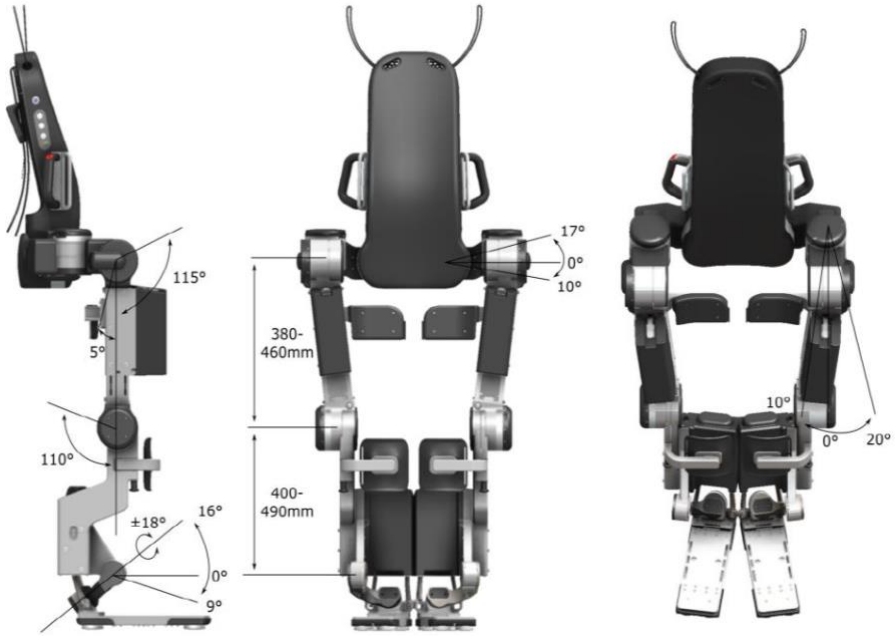

Figure 5: Angular amplitudes and mechanical adjustments of the V5 exoskeleton

Atalante exoskeleton is part of a system (Figure 6) composed of:

- **DataGen**: a dynamic adaptation of the algorithms is performed by a computation software running on a server called DataGen. It takes as input the patient measurements and computes a model of Atalante and the patient, as well as patient-specific trajectories for standing, sitting, walking, repositioning, and turning. All these outputs are transmitted to the exoskeleton.
- **AGUI (commercial name WanderTouch**): an application running on a portable device, called AGUI, offers a way to communicate with the exoskeleton using Bluetooth communication, as well as with DataGen using Wi-Fi. Data flows from AGUI to DataGen (patient measurements) and back from DataGen to AGUI to Atalante exoskeleton (algorithm outputs). AGUI also allows for real-time configuration and monitoring of a session, by fine tuning the gait pattern, and by selecting the level of assistance (ActiveGait) provided by Atalante to the patient while performing walking steps. Gait can be resistive (ActiveGait up to -25%), fully supported by the patient (ActiveGait 0%), assisted by Atalante at a chosen level per leg (ActiveGait up to 100%), or fully passive where Atalante provides all the necessary effort to perform the gait. Additional indicators are collected and available on AGUI per session and per patient.
- **Bluetooth dongle**: plugged in the back of Atalante and used to establish the communication between the AGUI and the exoskeleton.
- **Battery charger** for the removable battery packs that power the exoskeleton.
- **Dynamometric screwdriver** to adjust the thighs and legs.

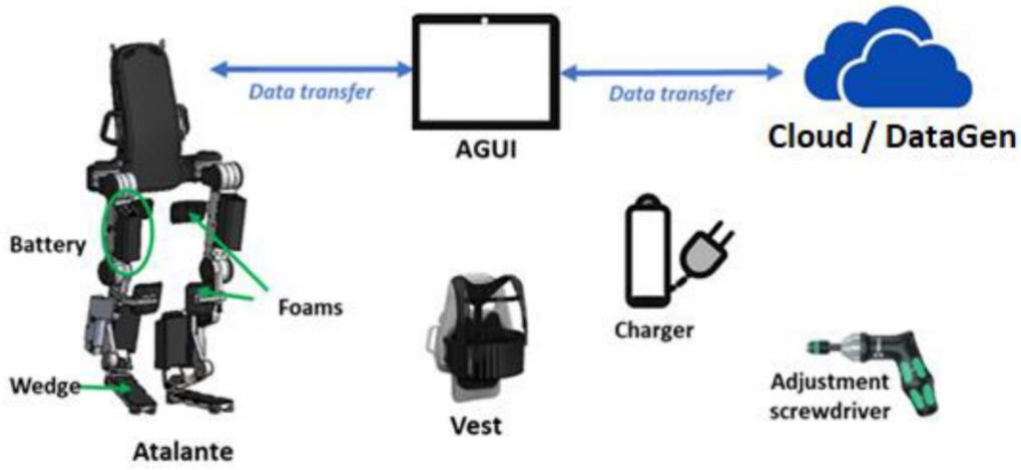

Figure 6: Atalante system

In addition, the Atalante system is used in combination with

- a seat with the following characteristics:
  - A minimal depth of 40 cm,
  - A minimal width of 65 cm,
  - A height in the range 40-55 cm,
  - Able to support at least 200 kg,
  - Be covered with a cushion.
- an electrical safety rail having the following characteristics:
  - Legally marketed medical device intended to support or lift people (compliance with ISO 10535 “Hoists for the Transfer of Disabled Persons - Requirements and Test Methods” standard or equivalent).
  - Stable and installed in accordance with the manufacturer instructions.
  - Able to support at least 200 kg.

The electrical safety rail and the seat are not distributed by Wandercraft. Only the swivel to link the safety rail to Atalante is provided with its protection. An example of safety rail without the protection is shown in Figure 6 below.

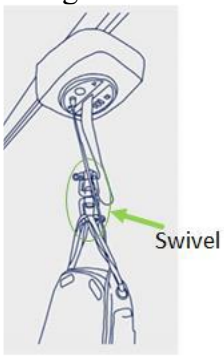

Figure 7: Atalante fixation to a safety rail without the protection

The exoskeleton is handled by the remote control, the “operator” interface located on the right-hand side of Atalante’s back and the sensor and an installation button located on Atalante's thighs at knee level at each side. The patient or the healthcare or the adapted physical activity professional select a mode or transition by pressing the relevant button. Then, the patient moves the bust; the sensor placed on the patient’s back detects the patient’s intention and trigger the movement.

The various modes and transitions are listed below:

- SITTING position: stable and comfortable sitting position.
- STANDING position: stable and comfortable standing position. The STANDING button also allows to switch to "exercise" mode, to stand up and to stop the motion.
- SITTING DOWN transition allows the patient to switch from standing up to sitting down.
- WALKING mode allows the patient to walk with Atalante.
- ROTATION mode allows the patient to turn with Atalante to the left or right.

- REPOSITION mode allows the patient to perform side and back steps with Atalante.
- INSTALLATION mode allows the patient to be transferred from his/her wheelchair to Atalante, or vice versa.

3.5.2 Regulatory approval

The Atalante system including its parts and accessories is considered as a Class IIa medical device following Rule 9, according to the annex VIII, according to the regulation (EU) 2017/745 and is covered by a EU certificate N° MDR 727 876 R000 issued by BSI on May 26th, 2021.

3.6 Intervention

The intervention will be delivered through the Neurorehabilitation Cemcat team.

Traditional Gait Rehabilitation training (tRHB):

Participants randomize to receive a tRHB, will be trained 3 times per week, with an intensity of 40 to 60 minutes, and duration over 8-weeks. Progression will occur by increasing the session intensity weekly.

A standardized protocol based on the usual clinical practice of physiotherapy in people with multiple sclerosis will be follow, according to patient characteristics, to design the sessions based on the patient objectives to be achieved, choosing the type of specific exercises to be performed based on the patient's condition and with the possibility of adapting them to their evolution during the twenty-four sessions.

PHYSIOTHERAPY TREATMENT PROTOCOL			
Goal	To re-educate the balance (sitting and standing).	To normalize upper limbs muscle tone	To improve the upper limbs muscular balance (MB)
Intervention	Trunk work on stable surface: anterior-flexion, lateralization and turns to make appear the straightening reactions (5 minutes)	Local cryotherapy application to the most affected muscles using cold-packs (15 minutes)	If BM = 1: isometrical exercises of deltoid, bicep, triceps, carpal extensors and carpal flexors (3 sets of 5 repetitions for each muscular group)
	Trunk work on instable surface (air cushion): anterior flexion, lateralization and turns to make appear the straightening reactions (5 minutes)	Inhibitory postural treatment in flexion, abduction and external rotation of humeral-scapula for the flexor patterns during cryotherapy application	If MB = 2: Weight-bearing and/or active-assisted exercises for deltoids, biceps, triceps, carpal extensors and carpal flexors (3 sets of 5 repetitions for each muscle group)
	Balance work with Bobath ball (5 minutes)	Passive mobilizations and myotendinous stretching of spastic musculature (5 minutes)	If MB= 3 : active-resisted exercises with ½ kg of deltoid, bicep, triceps, carpal extensors and carpal flexors (3 sets of 5 repetitions for each muscular group)
	Balance standing exercises at bars: antero-posterior and lateral load transfer, different support bases, and changing props and surfaces (10 minutes)	Pectoral and brachial biceps kneading and lats stretching (5 minutes)	If MB = 4: active-resisted exercises with 1 kg of deltoid, bicep, triceps, carpal extensors and carpal flexors (3 sets of 5 repetitions for each muscular group)

	Sitting		If MB = 5: active-resisted exercises with 2 kg of deltoid, bicep, triceps, carpal extensors and carpal flexors (3 sets of 5 repetitions for each muscular group)
	If Motor Assessment Scale score (MAS)≤ 4: sitting program low level		Propioceptive neuromuscular facilitation exercices (Kabat): diagonal on flexion-abduction-external rotation with shoulder pivot; diagonal on flexion-adduction-external rotation with elbow pivot (3 sets of 5 repetitions for each muscular group)
	If Motor Assessment Scale score (MAS) > 4: sitting program medium-high level		
	Standing		
	If Berg scale score ≤ 32: standing program low level		
	If Berg scale score 33-43: standing program medium level		
	If Berg scale score ≥ 44: standing program high level		
Goal	To re-educate the gait	To normalize lower limbs muscle tone	To improve the lower limbs muscular balance (MB)
Intervention	Spastic gait re-education exercises with emphasis on triple flexion during swing phase and initial heel strike to prevent foot drag (5 minutes)	Local cryotherapy application to the most affected muscles using cold-packs (15 minutes)	If BM = 1: isometrical exercises of major glutes, psoas, quadriceps, hamstrings and anterior tibial (3 sets of 5 repetitions for each muscular group)
	Walking on parallel bars with obstacles with emphasis on the triple flexion, on the dissociation of the shoulder and pelvic girdles and on the locking and unlocking of the knees (5 minutes)	Inhibitory postural treatment in abduction, external rotation and triple flexion for the extensor patterns during cryotherapy application	If MB = 2: discounted exercises and/or active-assisted of major glutes, psoas, quadriceps, hamstrings and anterior tibial (3 sets of 5 repetitions for each muscular group)
	Ataxic gait exercises (5 minutes)	Passive mobilizations and myotendinous stretching of spastic musculature (5 minutes)	If MB= 3: active-resisted exercises with ½ kg of major glutes, psoas, quadriceps, hamstrings and anterior tibial (3 sets of 5 repetitions for each muscular group)
	Incorporation of the most appropriate technical aid if necessary	Gastrocnemius kneading and adductors, hamstrings, sural triceps, quadriceps and psoas stretching (5-10 minutes for each limb)	If MB = 4: active-resisted exercises with 1 kg of major glutes, psoas, quadriceps, hamstrings and anterior tibial (3 sets of 5 repetitions for each muscular group)

	Running treadmill with speed and inclination according to tolerance (2-6 minutes)	Passive verticalization at standing or inclined plain to reduce the flexor musculature hypertonia (10 minutes)	If MB = 5: active-resisted exercises with 2 kg of major glutes, psoas, quadriceps, hamstrings and anterior tibial (3 sets of 5 repetitions for each muscular group)
	Weight-bearing walking (5 minutes).	Active verticalization at the shoulders or parallel bars in order to reduce the hypertonia of the flexor muscle (5-10 minutes)	Step from sitting to standing (3 sets of 5 repetitions)
	Vibratory stimulation in bilateral plantar zone (5 minutes)	Antero-posterior and lateral pelvic tilting exercises to encourage straightening reactions and reduce upper limbs hypertonia. (5-10 minutes)	Go up and down 20 stair's steps
		Pelvic and trunk rotations to decrease trunk extension hypertonia (5 minutes)	Active-resisted cycloergotherapy, resistance according to symmetry (10 minutes)
		Passive cycle ergotherapy adapting speed to tolerance and avoiding spasms during exercises (10 minutes).	

Atalante Gait training (RAGT):

Participants randomize to receive a Atalante gait training, will be trained 3-times per week, with an intensity of 40 to 60 minutes, and duration over 8-weeks. Progression will occur by increasing the session intensity weekly. Training intensity will be monitored and standardized using the Borg Rating of Perceived Exertion scale, and will progress from 'fairly light' to 'somewhat hard'. This prescription is consistent and appropriate for individuals with MS with mobility impairment and low fitness levels. Progression will occur by increasing the intensity of the session increase the number of steps in each session and decrease the level of assistance. Sessions will not exceed 60 minutes. We will record all training parameters.
Using the Atalante System, participants will be secured with the appropriately sized harness and attached to an overhead body-weight support system. Each session will begin with a 3–5-minute warm-up in the continuous passive mode (cadence ~40-45 steps/minute). The participant will then be transitioned into the adaptive training phase for practicing repetitive floor walking for up to 30 minutes. During this phase, the force produced by the robot is modulated to support the effort of the patient in producing a typical walking pattern.

Goals	Session nº/time	1 session. "Take in contact"/40 min	2nd and 3rd session / 30 min	2nd week / 30 - 35 min	3rd week / 35-40 min approx)
Improve the muscular balance of the deficient muscles of the lower limbs.	Robotmins assistance level (adapted to the patientmin s capacity)	100%	95%	90%	85%
Reeducate the balance in standing.		Take patient measurements and create the APP's profile	the accelerometer training	Walking with earlygait 5 for 10 min	Walking with earlygait mode 5min
Achieve a stable active bipedestation .		Learn how to change position from sitting (ST) to standing (SD) and back to ST.	Squat exercise 1 series x 5 rep.	Squat exercise 1 series x 10 rep.	The exoskeletonmin s controller is introduced to the patient in earlygait mode 5min.
Improve walking speed and gait pattern.		Learn how the accelerometer works from SD.	Trunk control exercises with balloon for 5 min	Trunk control exercises with ball 5min	Squat exercise 1 series x 10 rep.
Improve stability and safety while driving.		Learn how the movements and weight transfer work to activate the different functions/exercises .	Introduce the realgait mode walking (100% assisted)	Squat exercise 1 series x 5 rep.	Trunk control exercises with ball for 5 min.
Increase resistance to fatigue during walking.		Start by walking for 5 min with early-gait mode. Learn the turns, lateral steps, and "reverse march."	Walking with earlygait 5 for 5 min	Trunk control exercises with balloon for 5 min.	Squat exercise 1 series x 10 rep.
		Start trunk control exercises with a ball for 5 min, and learn how to squat and lateral transfers.		Realgait mode walk for 5 min (100% assisted)	Trunk control exercises with balloon 5min.
		A series of squats x 5 reps.		Realgait walk for 5 min. (Introduce assistance levels, 95% if the patient tolerates)	March with earlygait mode 5min dual task.
		Trunk control exercises with balloon 5min.			Realgait mode for 10 min (adjust levels of assistance based on tolerance

Activities

					and/or patient characteristics).
Session nº/time	4th week / 40 min	5th week / 40 min	6th week / 40 min	7th week / 40 min	8th week / 40 min
Robotmins assistance level (adapted to the patientmins capacity)	80%	75%	70%	65%	60%
Activities	Walking in earlygait mode for 5 min	Walking in earlygait mode for 10 min directed by patient	Walking in earlygait mode for 5 min directed by the patient	Walking in earlygait mode for 5 min directed by the patient	Walking in earlygait mode for 5 min directed by the patient
	Walking in earlygait mode for 5 min directed by the patient	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.
	Squat exercise 1 series x 10 rep.	Trunk control exercises with ball for 5 min.	Trunk control exercises with ball for 5 min.	Trunk control exercises with ball for 5 min	Trunk control exercises with ball for 5 min.
	Trunk control exercises with ball for 5 min.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.
	Squat exercise 1 series x 10 rep.	Trunk control exercises with balloon 5min.	Trunk control exercises with balloon 5min.	Trunk control exercises with balloon for 5 min	Trunk control exercises with balloon for 5 min
	Trunk control exercises with balloon for 5 min.	Squat exercise 1 series x 5 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.	Squat exercise 1 series x 10 rep.
	Walking in earlygait mode for 5 min + dual task.	Walking with earlygait 5min dual task.	Trunk control exercises with racket/basketbal l for 5 min.	Trunk control exercises with racket/basketbal l for 5 min.	Trunk control exercises with racket/basketbal l for 5 min
	Walking in earlygait mode for 10 min (We adjust assistance levels)	Walking in earlygait mode for 5 min (We adjust assistance levels)	Walking in realgait mode for 5 min, introducing dual task.	Walking in realgait mode for 10 min directed by the patient (We adjust assistance levels).	Walking in realgait mode for 10 min directed by the patient (We adjust assistance levels).

		Introduce use of the controller by the patient in realgait 5min.	Walking in realgait mode for 10 min directed by the patient (We adjust assistance levels).	Walking in realgait mode for 5 min, training dual task.	Walking in realgait mode for 5 min, training dual task.

4 Statistical considerations

The sample size calculation was based on the observed success rate (70%) in a previous evaluation of safety and performance of the self-balancing walking system Atalante in patients with spinal cord injury (i.e., 10 mWT with continuous walking mode)[15]
A sample size of 15 patients had been deemed necessary to demonstrate the performance with an alpha risk of 5% and a statistical power of 80% (Two-sided test).

Descriptive statistics (i.e., mean, standard deviation) will be calculated for quantitative data (demographics and clinical characteristics and outcome measures). Frequencies will be calculated for qualitative variables. For the proportions, binomial proportion confidences will be calculated using the Wilson formulae. Proportions will be compared using the Newcombe formulae
The Wilcoxon bilateral matched-pairs test will be used to compare repeated measurement on a single sample (mean, standard deviation).

Study data were collected and managed using REDCapelectronic data capture tools hosted at the Mayo Clinic. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources (Harris et al., 2009).

5 References

1. Feigin VL, Krishnamurthi R V., Theadom AM, et al. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol*. 2017;16(11):877-897. doi:10.1016/S1474-4422(17)30299-5
2. Otero-Romero S, Ramió-Torrentà L, Pericot I, et al. Onset-adjusted incidence of multiple sclerosis in the Girona province (Spain): Evidence of increasing risk in the south of Europe. *J Neurol Sci*. 2015;359(1-2):146-150. doi:10.1016/j.jns.2015.10.042
3. Kister I, Bacon TE, Chamot E, et al. Natural history of multiple sclerosis symptoms. *Int J MS Care*. 2013;15(3):146-158. doi:10.7224/1537-2073.2012-053
4. Comber L, Galvin R, Coote S. Gait deficits in people with multiple sclerosis: A systematic review and meta-analysis. *Gait Posture*. 2017;51:25-35. doi:10.1016/j.gaitpost.2016.09.026
5. Martin CL, Phillips BA, Kilpatrick TJ, et al. Gait and balance impairment in early multiple sclerosis in the absence of clinical disability. *Mult Scler J*. 2006;12(5):620-628. doi:10.1177/1352458506070658
6. Larocca NG. Impact of Walking Impairment in Multiple Sclerosis Perspectives of Patients and Care Partners Conclusions: Difficulty walking is a common impairment in people with MS, with adverse effects on the QOL of people with MS and care partners of a person with MS. *Patient*. 2011;4(3):189-201.
7. Motl RW, Sandroff BM, Suh Y, Sosnoff JJ. Energy cost of walking and its association with gait parameters, daily activity, and fatigue in persons with mild multiple sclerosis. *Neurorehabil Neural Repair*. 2012;26(8):1015-1021. doi:10.1177/1545968312437943
8. Pearson M, Dieberg G, Smart N. Exercise as a Therapy for Improvement of Walking Ability in Adults With Multiple Sclerosis: A Meta-Analysis. *Arch Phys Med Rehabil*. 2015;96(7):1339-1348.e7. doi:10.1016/j.apmr.2015.02.011
9. Drużbicki M, Guzik A, Przysada G, et al. Effects of Robotic Exoskeleton-Aided Gait Training in the Strength, Body Balance, and Walking Speed in Individuals With Multiple Sclerosis: A Single-Group Preliminary Study. Vol 102. The American Congress of Rehabilitation Medicine; 2021. doi:10.1016/j.apmr.2020.10.122
10. Yeh SW, Lin LF, Tam KW, Tsai CP, Hong CH, Kuan YC. Efficacy of robot-assisted gait training in multiple sclerosis: A systematic review and meta-analysis. *Mult Scler Relat Disord*. 2020;41:102034. doi:10.1016/j.msard.2020.102034
11. Bowman T, Gervasoni E, Amico AP, et al. What is the impact of robotic rehabilitation on balance and gait outcomes in people with multiple sclerosis? A systematic review of randomized control trials. *Eur J Phys Rehabil Med*. 2021;57(2):246-253. doi:10.23736/S1973-9087.21.06692-2
12. Xie X, Sun H, Zeng Q, et al. Do patients with multiple sclerosis derive more benefit from robot-assisted gait training compared with conventional walking therapy on motor function? A meta-analysis. *Front Neurol*. 2017;8(JUN):1-9. doi:10.3389/fneur.2017.00260
13. Sattelmayer M, Chevalley O, Steuri R, Hilfiker R. Over-ground walking or robot-assisted gait training in people with.multiple sclerosis: Does the effect depend on baseline walking speed and disease related disabilities? A systematic review and meta-regression. *BMC Neurol*. 2019;19(1):1-14. doi:10.1186/s12883-019-1321-7
14. Huynh V, Burger G, Dang QV, et al. Versatile Dynamic Motion Generation Framework: Demonstration With a Crutch-Less Exoskeleton on Real-Life Obstacles at the Cybathlon 2020 With a Complete Paraplegic Person. *Front Robot AI*. 2021;8(September):1-15. doi:10.3389/frobt.2021.723780
15. Kerdraon J, Previnaire JG, Tucker M, et al. Evaluation of safety and performance of the self balancing walking system Atalante in patients with complete motor spinal cord injury. *Spinal Cord Ser Cases*. 2021;7(1):1-8. doi:10.1038/s41394-021-00432-3
16. Kempen J, De Groot V, Knol DL, Polman CH, Lankhorst GJ, Beckerman H. Community walking can be assessed using a 10-metre timed walk test. *Mult Scler J*. 2011;17(8):980-990. doi:10.1177/1352458511403641
17. Gervasoni E, Jonsdottir J, Montesano A, Cattaneo D. Minimal Clinically Important Difference of Berg Balance Scale in People With Multiple Sclerosis. *Arch Phys Med Rehabil*. 2017;98(2):337-340.e2. doi:10.1016/j.apmr.2016.09.128

18. Sebastião E, Sandroff BM, Learmonth YC, Motl RW. Validity of the Timed Up and Go Test as a Measure of Functional Mobility in Persons with Multiple Sclerosis. *Arch Phys Med Rehabil*. 2016;97(7):1072-1077. doi:10.1016/j.apmr.2015.12.031
19. Vaney C, Blaurock H, Gattlen B, Meisels C. Assessing mobility in multiple sclerosis using the Rivermead Mobility Index and gait speed. *Clin Rehabil*. 1996;10(3):216-226. doi:10.1177/026921559601000306
20. Hobart JC, Riazi A, Lamping DL, Fitzpatrick R, Thompson AJ. Measuring the impact of MS on walking ability: The 12-item MS Walking Scale (MSWS-12). *Neurology*. 2003;60(1):31-36. doi:10.1212/WNL.60.1.31
21. Aymerich M, Guillamón I, Perkal H, et al. [Spanish adaptation of the disease-specific questionnaire MSQOL-54 in multiple sclerosis patients]. *Neurologia*. 2006;21(4):181-187. http://www.ncbi.nlm.nih.gov/pubmed/16832772
22. Lemay KR, Tulloch HE, Pipe AL, Reed JL. Establishing the Minimal Clinically Important Difference for the Hospital Anxiety and Depression Scale in Patients With Cardiovascular Disease. *J Cardiopulm Rehabil Prev*. 2019;39(6):E6-E11. doi:10.1097/HCR.0000000000000379
23. Kos D, Kerckhofs E, Carrea I, Verza R, Ramos M, Jansa J. Evaluation of the Modified Fatigue Impact Scale in four different European countries. *Mult Scler*. 2005;11(1):76-80. doi:10.1191/1352458505ms1117oa
24. Flachenecker P, Kümpfel T, Kallmann B, et al. Fatigue in multiple sclerosis: A comparison of different rating scales and correlation to clinical parameters. *Mult Scler*. 2002;8(6):523-526. doi:10.1191/1352458502ms839oa
25. Williams N. The Borg Rating of Perceived Exertion (RPE) scale. *Occup Med (Chic Ill)*. 2017;67(5):404-405. doi:10.1093/occmed/kqx063
26. Cleland BT, Ingraham BA, Pitluck MC, Woo D, Ng A V. Reliability and Validity of Ratings of Perceived Exertion in Persons With Multiple Sclerosis. *Arch Phys Med Rehabil*. 2016;97(6):974-982. doi:10.1016/j.apmr.2016.01.013
27. Grau-López L, Sierra S, Martínez-Cáceres E, Ramo-Tello C. Analysis of the pain in multiple sclerosis patients. *Neurol (English Ed*. 2011;26(4):208-213. doi:10.1016/s2173-5808(11)70043-8
28. Sipe JC, Knobler RL, Braheny SL, Rice GPA, Panitch HS, Oldstone MBA. A neurologic rating scale (NRS) for use in multiple sclerosis. *Neurology*. 1984;34(10):1368-1372. doi:10.1212/wnl.34.10.1368