O7_Protocollo dello studio

SPERIMENTAZIONE DI UN PERCORSO DIAGNOSTICO TERAPEUTICO ASSISTENZIALE PER LA RIABILITAZIONE INTEGRATA COGNITIVO-MOTORIA DEL PAZIENTE POST-ICTUS (MEMORI-NET)

CLINICAL TRIAL ON A THERAPEUTIC DIAGNOSTIC ROUTE FOR THE INTEGRATED COGNITIVE-MOTOR REHABILITATION IN THE POST-STROKE PATIENT (MEMORI-NET)

PROTOCOL SYNOPSIS

- **T0. Baseline and Screening (within 72 h after stroke)** (Done at the Neurology Ward)

  - **First neuropsychological assessment (reduced battery of questionnaires):**
    - Barthel Index (Mahoney and Barthel, 1965; Collin et al., 1988; 1 minute administration time)
    - NIH-NINDS (Marsh et al, 2016, about 10 minutes administration time)
    - The Montreal Cognitive Assessment (MOCA, about 10 minutes administration time)
    - The Frontal Assessment Battery (FAB, Iavarone et al., 2004, about 10 minutes administration time)

  - **Evaluation of inclusion/exclusion criteria**
  - **Written informed consent by patients or their relatives**
  - **Laboratory tests**
    - prognostic serum biomarkers of stroke outcome
    - genetic biomarker of responsiveness to rehabilitation therapy (BDNF polymorphisms)
    - pharmacogenomics for testing responsiveness to pharmacological pain therapies

- **T1. Baseline and Therapy start (1-2 weeks after stroke, at clinical stabilization)**

(Done either at the Neurology Ward or at rehabilitation clinics, based on patient's conditions and local organization)

  - **Full battery of cognitive questionnaires:**
    - LANGUAGE: naming and word comprehension tasks of the Esame Neuropsicologico per l'Afasia (ENPA, Capasso et al., 2001, 5-10 min. administration time);
    - VISUOSPATIAL ATTENTION: star cancellation test of the Behavioural Inattention Test Battery (BIT, Spinazzola et al, 2010, 1-3 min. administration time);
    - WORKING MEMORY: forward and backward Digit and Corsi Span test (Monaco et al., 2013, 10 minutes administration time)
    - LONG-TERM MEMORY: short recognition memory test for words and faces (Warrington, 1996; 10 minutes administration time);
    - EXECUTIVE FUNCTIONS:
      - Trail Making Test (both TMT-A and TMT-B, Giovagnoli et al., 1996; 10 min. administration time);
      - Brixton (Spitoni et al., 2017, about 10 min. administration time);
      - Stroop test (short version, Cafarra et al., 2002, 10 min. administration time);
      - Tower of London (Krikorian et al., 1994, 15 min. administration time)

  - **Assessment of pain with the following scales:**
    - Visual Analog Scale (VAS), (McCormack et al, 1988; <1 minute to complete)
    - McGill patient-reported questionnaire (Melzack et al. 1987; 10 min. administration time)

- Neuropathic Pain Symptom Inventory (NPSI) (Bouhassira et al. 2004; 2 minutes administration time)
- Sensory tests of mechanical allodynia, touch and temperature sensations tests (Cruccu et al. 2010).
- Dolorimeter measure on trigger points

- **Assessment of motor disabilities with the following scales:**
  - Barthel Index (Mahoney and Barthel, 1965; Collin et al., 1988; <1 minute)
  - NIH-NINDS (Marsh et al, 2016, about 10 minutes administration time)
  - Rankin modified scale (Rankin, 1957; Bonita and Beaglehole, 1988, 5 minutes for administration)
  - Trunk Control Test TCT (Duarte, Verheyde; 10 minutes)
  - Fugl-Meyer Assessment scale for the upper and lower extremity (Fugl-Meyer., 1975)
  - MIQ-Revised Second version scale (MIQ-RS) for the ability to image movements [Butler et al. 2012].
  - Gait test with dual tasks (walking test) (Marusic et al., 2015)
  - Senior fitness test with Grip strength (Paravlic et al., 2017)
  - Tensomyography (TMG) (Simunic et al., 2011)
  - Ashworth 5 points scale for spasticity (Charalambous, 2014)

- **Second evaluation of inclusion/exclusion criteria and randomization and assignment of the rehabilitation Protocol A (experimental) or B (control)**

- **Laboratory tests**
  - serum biomarkers of responsiveness to rehabilitation

- **Other clinical evaluations:**
  - Quantitative EEG with power spectrum analysis (PSD)

- **Rehabilitation Protocol A (<u>experimental group</u>):**

COGNITIVE REHABILITATION
- Standard occupational therapy
- Standard speech therapy (only if there is aphasia, dysphagia or dysarthria)
- Cognitive rehabilitation exercise 1- Updating
- Cognitive rehabilitation exercise 2- Manipulation
- Cognitive rehabilitation exercise 3- Everyday Planning
- Cognitive rehabilitation exercise 4- Action Sequences
- Cognitive rehabilitation exercise 5- Go-no-go Task
- Cognitive rehabilitation exercise 6- Modified Stroop Tasks
- Cognitive rehabilitation exercise 7- Task switching
- Cognitive rehabilitation exercise 8- Barrage
- Cognitive rehabilitation exercise 9- Oddball Task
- Cognitive rehabilitation exercise 10- Dual Task

MOTOR REHABILITATION
- Standard physical exercise
- Standard Physiotherapy
- Standard Muscle Electrostimulation protocols
- Virtual imagery motor rehabilitation with NEUROFEEDBACK of the hand or the limbs

- **Rehabilitation Protocol B (<u>control group</u>):**

COGNITIVE REHABILITATION
- Standard occupational therapy

- Standard speech therapy (only if there is aphasia, dysphagia or dysarthria)

MOTOR REHABILITATION
- Standard physical exercise,
- Standard Physiotherapy
- Standard Muscle Electrostimulation protocols

- **T2. Therapy checkpoint n.1 (4 weeks from T1)**

  - **Same motor and cognitive assessments as in T1**
  - **Same Rehabilitation protocols A and B as T1**

- **T3. Therapy checkpoint n.2 (at the end of rehabilitation = 5-12 weeks from T1)**

  - **Same motor and cognitive assessments as in T1**
    - The Montreal Cognitive Assessment (MOCA)
    - The Frontal Assessment Battery (FAB)
  - **Same Rehabilitation protocols A and B as T1 until end of therapy**
  - **Laboratory tests**
    - serum biomarkers of responsiveness to rehabilitation

- **T4. Therapy Follow-up (at 3 months from the end of rehabilitation):**

  - **Same assessment as T1**
  - The Montreal Cognitive Assessment (MOCA)
  - The Frontal Assessment Battery (FAB)

SCHEDULE OF TESTS

TEST NAME	T0	T1	T2	T3	T4
The Montreal Cognitive Assessment (MOCA)	X			X	X
The Frontal Assessment Battery (FAB)	X			X	X
Barthel Index	X	X	X	X	X
NIH-NINDS	X	X	X	X	X
Naming and word comprehension tasks		X	X	X	X
Star cancellation test BIT		X	X	X	X
Forward and backward Digit		X	X	X	X
Corsi Span test		X	X	X	X
Short recognition memory for words and faces		X	X	X	X
Trail Making Test (TMT-A and TMT-B)		X	X	X	X
Brixton		X	X	X	X
Stroop test – short version		X	X	X	X
Tower of London		X	X	X	X
McGill questionnaire		X	X	X	X
Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)		X	X	X	X
Neuropathic Pain Symptom Inventory (NPSI)		X	X	X	X
Analysis of mechanical allodynia		X	X	X	X
Mechanical allodynia, touch and temperature sensations tests		X	X	X	X
Rankin modified scale		X	X	X	X
Trunk Control Test TCT		X	X	X	X
Fugl-Meyer Assessment		X	X	X	X
MIQ-Revised Second version scale (MIQ-RS)		X	X	X	X
Gait test with dual tasks (walking test)		X	X	X	X
Senior fitness test with Grip strength		X	X	X	X
Tensomyography (TMG)		X	X	X	X
Ashwarth 5 points scale for spasticity		X	X	X	X
Quantitative EEG with power spectrum analysis (PSD)		X	X	X	X
Genetic markers of responsiveness to rehabilitation therapy (BDNF polymorphisms)	X				
Pharmacogenomics for responsiveness to pharmacological pain therapies	X				
Prognostic serum biomarkers of stroke outcome	X			X	
Serum biomarkers of responsiveness to motor/cognitive rehabilitation		X		X	
Cognitive rehabilitation exercises		X	X	X	

Standard cognitive rehabilitation exercises		X	X	X	
Standard motor rehabilitation exercises		X	X	X	
Neurofeedback		X	X	X	

Title of the study protocol:

CLINICAL TRIAL ON A THERAPEUTIC DIAGNOSTIC ROUTE FOR THE INTEGRATED COGNITIVE-MOTOR REHABILITATION IN THE POST-STROKE PATIENT

Acronym: **MEMORI-net**

Synopsis:

Background. With more than 1 million new cases/year, stroke represents one of the most urgent causes of intellectual and motor disability in European countries. Stroke can affect still productive persons, and therefore has a profound impact on the healthcare system, the families and the whole local economy. Although stroke is an acute vascular injury of the brain, it may result in disabilities in the whole body, which require multidisciplinary practices of rehabilitation. Indeed, scientific studies have demonstrated that the combination of cognitive and motor training produces the best results in terms of patient recovery, however there is a lack of well-defined rehabilitation protocols that include these two dimensions.

Aims. This project aims at designing and testing a new Pathway of Diagnostic and Therapeutic Assistance for an integrated cognitive and motor rehabilitation protocol. In particular, the proposed study aims to compare the effectiveness of a Protocol A which combines cognitive rehabilitation in the domains of memory and attention, motor rehabilitation performed by virtual reality, and standard physiotherapy, compared to a Protocol B which offers a rehabilitation with only the standard physiotherapy.

Study design. **Protocol A (experimental group):** The combined motor and cognitive rehabilitation protocol will be composed by different training modules that are designed to stimulate the still-preserved neuronal circuits to perform the functions that were damaged by stroke. Cognitive rehabilitation modules will focus mainly on the rehabilitation of attention-related processes and memory, since these functions are the most frequently affected by stroke. Motor rehabilitation will occur by looking at virtual representation of movements and then forming the mental image of what just observed. The learning process will be supported by the use of electroencephalography (EEG) to detect the electrical activity of the brain. This allows a continuous feedback (called neurofeedback) on the efficiency of cognitive rehabilitation exercises. Patients will learn how to produce EEG waves that conforms to real motor execution and will become able to simulate real body movements, without a physical commitment that, because of brain damage, might not yet be feasible or not satisfactorily viable. The above training modules will be given in association with standard physiotherapy consisting in neuromuscular electrical stimulation (NMES) combined with voluntary movement training, which is one of the most effective interventions for motor rehabilitation after stroke. It affects motoneuron terminals projecting to skeletal muscle fibers. Our protocols will use rectangular, fully compensated, bi-phasic impulses with variable stimulation patterns on individual bases. **Protocol B (control group):** The standard therapy consists in motor rehabilitation including neuromuscular electrical stimulation (NMES) combined with voluntary movement training, assisted by physiotherapists and cognitive rehabilitation including occupational therapy and speech therapy, when needed.

Expected results. Treatment will be considered beneficial if treatment could lead to changes in the following endpoints:

1. COGNITIVE FUNCTIONS: Patients scoring at the Montreal Cognitive Assessment (MOCA) should reach at least 26/30 (normal range) and The Frontal Assessment Battery (FAB) score should improve up to 13/18 or more;
2. MOTORS FUNCTIONS: An average improvement of about 3 points in the Fugl-Meyer Assessment (FMA) scores for Upper and Lower Extremity and for Cordination/Speed is expected; The MIQ-RS is a 14-item questionnaire that rates one's ability to imagine and consists of 7 visual and kinesthetic items. Total MIQ-RS score and its kinesthetic and visual subscores will be higher of about 5 points for the visual motor image and of 3 points for kinesthetic motor image. BARTHEL INDEX at least 1 point in any of the subscales; NIH-

NINDS at least 1 point in any of the subscales; TCT at least 1 point in any of the subscales; RANKIN MODIFIED SCALE at least 1 point in any of the subscales.
3. PAIN INDEX, improvement in at least one of the following pain questionnaire scales: VAS; McGill, NPSI (score of any of these scales improved by at least 30%)
4. BIOMARKERS: for pain at least 1 biomarker decreased by 10%; brain at least 1 biomarker increased by 10%; muscle at least 1 biomarker increased by 10%, immune function/inflammation at least 1 biomarker decreased by 10%.

Keywords: Stroke, motor impairment, cognitive impairment, physical rehabilitation, cognitive therapy, chronic pain, brain plasticity biomarkers, motor activity biomarkers, immune system biomarkers, pain biomarkers, cytokines, myokines, neurotrophic factors.

BACKGROUND:

With an incidence of 1.1 million new cases per year stroke was identified as the first leading cause of neurological disability and in most European countries, stroke is the second or third most common cause of death [Truelsen et al., 2006; Warlow et al. 2008; Bonita et al. 2004]. Following stroke, patients typically enter rehabilitation programs (i.e. physical therapy, occupational therapy, etc.) to address a multitude of motor, emotional and cognitive deficits [Taylor & Francis 2006; Doppelmayr et al. 2007]. Traditionally, stroke primarily marks functional motor impairments [Langhorne et al. 2009].

Cognitive impairment commonly occurs in the acute phase post-stroke and may persist with over half of all stroke survivors experiencing some form of long-term cognitive deficit (Mellon et al., 2015). Post-stroke cognitive impairment frequently occurs, in 20% up to 80% of patients, with differences depending on country, race and diagnostic criteria [Sun et al., 2014]. Age and education level highly modulate the cognitive impairment risk, with an exponential increase in the prevalence of post-stroke cognitive decline in patients' older than 65 years [Gorelick et al., 2011]. The most common types of cognitive deficits arising from stroke are disturbances of attention, language syntax, delayed recall and executive dysfunction affecting the ability to analyze, interpret, plan, organize, and execute complex information (Jokinen et al., 2006; Sachdev et al., 2004). Because there is no standardized definition for cognitive impairment and no standardized assessment tools for testing cognition after stroke, prevalence rates and patterns of impairment often vary among studies involving attention, spatial ability, memory and language. Along with impairment in the above mentioned cognitive domains, the occurrence of executive disorders is increasingly being acknowledged as a recurring consequence not only of anterior, but also of posterior and subcortical stroke, which could interfere with the process of rehabilitation and recovery [Jankowska et al., 2017] and may play a critical role in predicting functional recovery. Given the considerable impact of cognitive impairment on functional outcome, it is of primary importance to identify acute predictors of these cognitive and functional outcomes with a view to tailoring rehabilitation programs and improving clinical care.

Pain treatment is a necessary component of patient's management and rehabilitation, as important co-morbidity of stroke. Neuropathic pain after stroke can be caused by CNS lesions, in the somatosensory tracts (central post-stroke pain). It is poorly recognized and might incur with several weeks of delay from the acute event. An open issue is how effectively assessing pain in cognitive impairment conditions and how to effectively treat it together along other co-morbidities. It is interest for the best management of patients and for the rehabilitation outcomes, to clarify the occurrence of pain symptoms and their origin, and to timely identify patient-related factors that may influence the effectiveness of pharmacological pain treatments. For the best pain assessment, we will use a battery of different pain-specific tests based on questionnaire and direct evaluation made by neurophysiologists accordingly with the most recent guidelines, including tests for the presence of mechanical allodynia and preserved nociceptive and sensorial function with temperature discrimination (O'Donnell et al., 2013).

Biomarkers measured from body-fluid in post-stroke patients have reached an outstanding and remarkable revision in the very recent years. Besides the diagnostic and prognostic role of some inflammatory markers,

like such as C-reactive protein (CRP), IL-6, IL-1β and TNFα other molecules and biological factors have been added to the list, including tissue derived cytokines, growth factor-like molecules, hormones, and microRNAs (Gandolfi et al., 2017). These include for instance, proteins released by skeletal muscle, especially during physical exercise like the myokines Irisine, Myostatine, Follistatine, C-terminal Agrin Fragment (CAF22), which are considered biomarkers of the efficacy of motor rehabilitation and may promote brain plasticity and neuroprotection (Coelho et al. 2016; Lightfoot and Cooper, 2016). On the other hand, brain-specific markers were associated with reactivation of neuronal plasticity after stroke and include the neurotrophin members BDNF, NT-3, NGF, or the GDNF and IGF-1. The neurotrophic and neuroprotective effects of these factors are likely to act in concert with vascular factors considered indicators of blood-brain barrier integrity and reactivators of angiogenesis such as VEGF, PDGF, MMP-9 (Jicking and Sharp, 2015). In addition, because of the increasing attention given to pain management, several studies have pointed to the potential usefulness of serum biomarkers associated with pain sensitivity such as CGRP, Serotonin, Substance-P, Proenkephalin A, Neuropeptide-Y (Jicking and Sharp, 2015; Gandolfi et al., 2017).

Rehabilitation training is the most effective way to reduce motor and cognitive impairments in stroke patients. Improvement probably occurs through a complex combination of spontaneous and learning-dependent processes including: restitution, substitution, and compensation (Kwakkel et al., 2004; Langhorne et al., 2011). Interdisciplinary complex rehabilitation interventions represent the mainstay of post-stroke care (Langhorne and Legg, 2003; Langhorne et al., 2011). Neurofeedback (NFB) therapy is becoming more and more recognized as a treatment modality that can help the brain after stroke to repair itself [Buch et al. 2009; Ang et al. 2009; Nelson 2007; Sharma et al. 2006]. NFB is capable of bypassing the normal motor output of neural pathways and directly translating brain signals into commands for control of external devices [Hammond 2006; Pfurtscheller et al. 2009]. NFB systems of this type usually estimate the patient's motor intention from the changes in brain activity over primary sensorimotor cortex and display them through visual feedback [Hammond 2006; Nelson, 2007]. Using this information the patient can consciously adapt his/her brainwave activity to reach targeted training thresholds. Previous studies using NFB technology yielded some plastic changes in the SMR and improvement in motor performance [Pichiorri et al. 2011; Mattia et al. 2012]. Those systems are thus expected to help guide cortical reorganization by motor learning, and to make neurorehabilitative approaches more effective. Given that classic, pharmacological and experimental treatments, including NFB and non-invasive brain stimulation (NIBS), might provide significant effects independently and with the rationale that they could work in a synergistic fashion, we believe that a combined therapy can lead to better outcomes than one or the other alone.

CLINICAL QUESTION AND RATIONALE:

Clinical question: This project aims at designing and testing a new Pathway of Diagnostic and Therapeutic Assistance for an integrated cognitive and motor rehabilitation protocol. In particular, the proposed study aims to compare the effectiveness of a Protocol A which combines cognitive rehabilitation in the domains of memory and attention, motor rehabilitation performed by virtual reality, and standard physiotherapy, compared to a Protocol B which offers a rehabilitation with only the standard physiotherapy.

General Rationale: Rehabilitation starts immediately after the acute phase of stroke, within the stroke-units at neurology clinics. Following this phase, patients are discharged from stroke-units and addressed to rehabilitation centers. Discharge record typically includes a multidimensional assessment of the clinical stability according to the Modena 2000 criteria, language disability test, dysphagia and sphinteric disturb evaluation, analysis of the socio-familiar context, psychological assessment in particular for depression. However, depending on the geographical location and the degree of disability, patients are addressed for rehabilitation therapies to various destinations such as public hospitals, private clinics, territorial structures and specialized rehabilitation centers, private thermal baths or clinics, and often to a combination of them. Unfortunately, among these centers there are large differences in the protocols used for the assessment of the patients at the entry and during the subsequent monitoring of rehabilitation, thus making difficult the transfer from one station to another, and often jeopardizing the efficacy of the rehabilitation treatments.

Technical Rationale: New motor rehabilitation methods, based on the recovery of the mental representation of the movement (Action Observation AO and Motor Imagery MI) can facilitate patients in the process of relearning motor strategies. The AO and MI methods are based on the use of neural pathways of the voluntary control of action that bypass the neural circuits altered by the brain lesion. These neural pathways, include the dorsal circuit of the striatum that is still relatively preserved in stroke patients. This protocol will be designed to help the patients to learn how to use the still preserved neuronal circuits to carry out the functions that are damaged by stroke, just visualizing on a computer monitor a virtual representation of the movements (in a movie or a cartoon) and then closing the eyes and forming a mental image of the movements just observed. This learning process can be enormously facilitated if the patient wears Electro-Encephalo-Gram (EEG) sensors and headsets that are connected to the computer on which the patients is making exercise. These sensors have from 2 to 16 recording EEG channels and can give to the patient a continuous feedback (called neurofeedback) on the efficacy of the cognitive rehabilitation exercises and provides suggestions on how to improve the patient performance. These systems will be used for in-hospital and at home rehabilitation, and will have different interfaces modalities, i.e. patients, caregivers and clinicians.

The ICT-based platform will cover several aspects of proper rehabilitation. First, with the help of neurofeedback we will assess patient's capability to perform simple attentional tasks during which the duration and the level of involvement in that specific task will be monitored. Secondly, patient will learn throughout several difficulty steps how to produce EEG waves that correspond to the real motor execution and arrive at moving objects on the screen with the only strength of the mind. Finally, when patient will reach a certain level of commanding the movement in the virtual environment, the AO and MI of different motor-cognitive tasks will be performed. In order to obtain the patient satisfaction as well as the level of involvement in the training/rehabilitation program, the ICT-platform will offer a self-assessment questionnaire which will be filled by clicking at one evaluation scale, at the end of each training session.

The diffusion of mobile technologies and the availability of new digital applications (APP) designed to stimulate cognitive skills have added new opportunities for post-stroke rehabilitation. These APPs, available on iOS, Android or iPad, offer games, puzzles etc able to stimulate cognitive and motor skills of a device user. We will create 10 APP for rehabilitation exercises commonly done with pen and paper.

Assessment questionnaires including cognitive, motor and pain components will be selected considering the best gold standards in the field. The questionnaires will be adopted in double language, in order to fulfill demands of Italian/Slovenian patients. Integration of data from cognitive, motor and pain components will generate an innovative and integrated tool for clinical practices for the assessment of the patient's state, useful to organize the proper therapy. Impairment of cognitive functions is a very frequent complication of stroke, often the predominant clinical feature. Cognitive deficits can increase disability, affect functional recovery and therefore reduce the quality of life after stroke.

One part of the study is dedicated to the definition of a protocol for the identification of the grade of the injury, cognitive decline, or motor impairment and pain using validated biomarkers detectable in human body fluids. These biomarkers will be also used to stratify patients and to test the effectiveness of specific pharmacological and rehabilitation treatments. Single patient monitoring via biomarker analysis represent a strong need to achieve personalized clinical treatments and increase their efficacy.

OBJECTIVES OF THE STUDY:

MEMORI-net's objective is to investigate whether a combined motor and cognitive rehabilitation treatment can represent a valid option to improve the daily quality of life of post-stroke patients. Stroke-associated comorbidities have a remarkable impact on patients and families, and the goal of improving these symptoms represents a worthy endeavour while awaiting a more definitive cure.

Study endpoints:

As far as the Cognitive Rehabilitation, participants will be alternately assigned into either the new computer-assisted cognitive training (CAC) or control group. Concerning the **COGNITIVE ENDPOINTS,** treatment group will focus on working memory, planning, and top-down components of executive functions (voluntary control over automatic processes). Pre/post changes are expected to be either similar or superior in the CAC than in the control group. Concerning **MOTOR ENDPOINTS,** treatment group will focus on motor recovery following virtual imagery training through a neurofeedback (NFB) therapy. Pre/post changes are expected to be either similar or superior in the NFB than in the control group. Concerning **PAIN ENDPOINTS,** it is expected that after the combined CAC+NFB training, patients will report a reduction in pain measurement scales. The improvement in these three domains will be associated with significant pre/post-treatment changes in **SERUM BIOMARKERS as surrogate endpoints**.

STUDY POPULATION:

In order to evaluate the clinical efficacy of a combined motor and cognitive rehabilitation protocol, we plan to perform a randomized, non-blinded, multicentric, non-commercial clinical study. A total of 120 post-stroke adult patients with no age limits will be enrolled in the study.

Three neurology clinics, two located in the public Italian hospitals of Portogruaro (VE) and Trieste (TS) and one in the public Slovenian hospital in Izola (KP) will be involved in the study. All of them are tertiary care Hospitals with a long standing experience in diagnosis and treatment of stroke patients and can provide a multidisciplinary intervention for patients with neurological diseases. In addition, three other public hospitals with rehabilitation centers, two in Italy (Trieste and Portogruaro) and one in Slovenia (Sežana) will be involved in the therapeutic intervention. All these six centers are Partners or Associated Partners of the project MEMORI-net sponsored by the Italy/Slovenia Interreg V-programme.

Patients will be recruited by the three neurology clinics by an open call recruitment. Patients who entered the clinical centers following a stroke, are eligible for selection and, once stabilized will start rehabilitation and will remain into the study for up to 6 months (from stroke to the last follow-up visit).

Patients will be considered to have completed the study after having conducted the last follow-up visit. Patients can withdraw from the study anytime as per their decision or can be withdrawn by investigator for lack of compliance or safety reasons. A patient who withdraws from the study before completion of period T2, can be replaced (excluding withdrawals for safety reasons).

INCLUSION CRITERIA:

1. Type of injury

- Patients will be included in the MEMORI-net study if they present a unilateral ischemic episod.

2. Post-stroke phase:

- participants will be in the post-stroke sub-acute phase (i.e in <7-15 days post-stroke).

3. Abilities:

- participants will be able to follow verbal instructions and to communicate,
- participants will be able to perform all the programmed tests.

4. Age:

- adults, no-age limits

5. Gender:

- all gender

6. Region of lesions:

- individuals suffered from unilateral hemisferic lesions

7. Expected deficit/s (one or more of the following):

- difficulty raising the hand/s,
- all degrees of paresis
- attentional and/or executive dysfunction

8. Consensus:

- only those patients who gave their written consent to participate in the study

EXCLUSION CRITERIA:

- hemorrhagic stroke
- other serious medical conditions
- pacemaker or other implanted stimulators
- history of non-controlled seizures
- severe cognitive deficits
- severe aphasia
- unilateral spatial neglect
- never attended a school
- had experienced NFB within the past year
- patients who did not give their written consent to participate in the study

STUDY DESIGN:

We designed a prospective, randomized, non-blinded study to evaluate the efficacy in post-stroke patients of a rehabilitation **protocol A** which combines virtual motor training and intensive cognitive therapy associated with standard physiotherapy and occupational + speech therapy, with respect to a control rehabilitation **protocol B** comprising standard physiotherapy and occupational + speech therapy.

The study will include the following 5 phases:

-T0 Baseline (within 72 h after stroke): subjects will be considered for the study if fulfilling inclusion criteria and then included in the study. The first neuropsychological assessment will take place within the first 72 hours after the event. At baseline, a reduced battery of questionnaires (see Intervention) will be administered to the patients by the neurology ward[1] (see Intervention). Patients will be randomized into 2 sub-groups, one with the combined protocol A (treatment group) and one with the normal protocol B (control group). Serum levels of biomarkers to predict treatment outcomes and stratify patients will be determined. In addition, genetic biomarkers of responsiveness to rehabilitation therapy (including BDNF polymorphisms) and pharmacogenomics for testing responsiveness to potential pharmacological therapies will be assessed for all participants.

-T1 Therapy start (1-2 weeks after stroke, at stabilization): At the beginning of the rehabilitation therapy a full battery of questionnaires (see Intervention) will be filled in by all patients. Laboratory tests for serum biomarkers of muscle signaling, neuronal plasticity/damage and inflammation will done in order to establish the baseline for a subsequent monitoring of the treatment efficacy. The level of motor disability will be assessed through a battery of scales that will be administered by the physician (see Intervention). Patients'

[1] **Neurological wards**: Cattinara-Trieste, Izola (KP), Portogruaro (VE)

cognitive functions will be assessed by psychologists in the neurology or in the rehabilitation ward on the day before the rehabilitation start, using a full battery of tests for the area Language, Visuospatial attention, Working memory, Long-term memory, Executive functions, Sensorial dysfunctions and Pain. Only participants who showed an attentional and/or executive dysfunction will be enrolled in the rehabilitation program whereas if they show severe aphasia or neglect they will not take part to the study.

Patients will be alternately assigned to either the experimental group or to the control group.

Cognitive rehabilitation in the Control group will include occupational therapy and speech therapy, when needed. Cognitive rehabilitation in the Experimental group will focus on working memory, planning, and top-down components of executive functions (voluntary control over automatic processes).

Motor rehabilitation in the control group will include electromyostimulation and standard physiotherapy with increasing intensity depending on the patient's need. Experimental group will undergo computer assisted neurofeedback training (see Intervention) in addition to occupational therapy, electromyostimulation and standard physiotherapy.

- T2 Therapy checkpoint n.1 (4 weeks from T1): All tests for cognitive functions administered at T1 will be re-administered at T2 (after 4 weeks of the rehabilitation program). Patients will either stop or continue until T2 the cognitive and motor training program as at T1, depending on their need.

- T3 Therapy checkpoint n.2 (5-12 weeks from T1): All of the tests for cognitive functions administered at T1 will be re-administered at T3 (after 8 weeks of the rehabilitation program) to the patients enrolled in the rehabilitation program which will end at T3. Serum levels of biomarkers for treatment efficacy will be determined.

- T4 Therapy follow-up (3 months from T3): All of the tests for cognitive functions administered at T1, and MOCA and FAB tests will be re-administered at T4 to the patients enrolled in the rehabilitation program

INTERVENTION

Neuropsychological assessment instruments (for both treatment and control groups)

At T0 the **cognitive disabilities** will be assessed in patients through the following scales:

- The Montreal Cognitive Assessment (MOCA, about 10 minutes administration time)

- **The Frontal Assessment Battery** (FAB, Iavarone et al., 2004, about 10 minute administration time)

At T1, T2, T3 and T4 the **cognitive functions** will be evaluated in patients with the following tests that will be administered by psychologists:

– LANGUAGE will be assessed by administering the naming and word comprehension tasks of the Esame Neuropsicologico per l'Afasia (ENPA, Capasso et al., 2001, about 5-10 min. administration time); scoring will be based on the number of correct responses.

Naming Task (Min-Max scores)**:**

Verbs (Oral 0-10; Written 0-5)

Names (Oral 0-10; Written 0-5)

Colours (Oral 0-10)

Comprehension Task (Min-Max scores)**:**

Words (Auditory 0-20; Visual 0-20)

Sentences (Auditory 0-14; Visual 0-14)

– VISUOSPATIAL ATTENTION will be evaluated with the Star cancellation test of the Behavioural Inattention Test Battery (BIT, Spinazzola et al, 2010, about 1-3 min. administration time); scoring will be based on the analysis of reaction times (RTs), perseverations, total items cancelled, left/right differences (Min-Max Scores: 0-54; Ratio Neglect-Side Omissions/Total Omissions >0.75 Pathologic)

– WORKING MEMORY will be assessed through the Forward and Backward Digit test and the Corsi Span test (Monaco et al., 2013, about 10 minutes administration time); scoring will be based on the number of correct item sequences (Min-Max Scores: 0-9);

– LONG-TERM MEMORY will be tested with the following tests and scoring will be based on accuracy and/or RTs accordingly to each test: Short recognition memory test for words and faces (Warrington, 1996 about 10 minutes administration time); scoring will be based on the number of correct responses (Min-Max Scores: Words 0-25; Faces 0-25);

– EXECUTIVE FUNCTIONS will be tested with the following tests: Trail Making Test TMT-A and TMT-B tests (Giovagnoli et al., 1996) about 10 min. administration time; Brixton (Spitoni et al., 2017, about 10 min. administration time; score: total number of errors = maximum 54); Stroop test (short version, Cafarra et al., 2002, about 10 min. administration time, Min-Max Scores: Total Number of Errors: Words 0-30; Colors 0-30; Interference C/W 0-30); Tower of London test (Krikorian et al., 1994, about 15 min. administration time, Min-Max Scores: 0-36).

Motor disability assessment instruments (for both treatment and control groups)

At T0 the **motor disabilities** will be assessed in patients through the following scales:

- **Barthel Index** (Mahoney and Barthel, 1965; Collin et al., 1988; <1 minute administration time)
- **NIH-NINDS** (Marsh et al, 2016, about 10 minutes administration time)
- **TCT** (Duarte, Verheyde; 10 minutes administration time)
- **Rankin modified scale** (Rankin, 1957; Bonita and Beaglehole, 1988, 5 minutes administration time)

At T1, T2, T3 and T4 the **motor disabilities** will be assessed in patients through the following scales that will be administered by the physician:

- **Barthel Index** (Mahoney and Barthel, 1965; Collin et al., 1988; <1 minute administration time)
- **NIH-NINDS** (Marsh et al, 2016, about 10 minutes administration time)
- **TCT** (Duarte, Verheyde; 10 minutes administration time)
- **Rankin modified scale** (Rankin, 1957; Bonita and Beaglehole, 1988, 5 minutes administration time)
- **The Frontal Assessment Battery** (FAB, Iavarone et al., 2004, about 10 minute administration time)
- **Gait test with dual tasks** (walking test, Marusic et al., 2015, about 10 minutes administration time),
- **Senior fitness test with Grip strength** Paravlic et al., 2017, about 20 minutes administration time),
- **Tensomyography** (TMG Simunic et al., 2011 about 20 minutes administration time),
- **Ashworth 5 points scale for spasticity** Charalambous, 2014, about 5 minutes administration time),
- **Fugl-Meyer Assessment** scale for the upper and lower extremity (Fugl-Meyer., 1975, about 60 minute administration time). The four domains assessed by Fugl-Meyer scale will be motor function (maximum score in upper limb = 66; maximum score in lower limb = 34), sensory function (maximum score = 24), balance (maximum score = 14), range of motion of joints (maximum score = 44). To perform the test, a tennis ball and a small spherical shaped container and knee hammer will be need.
- **MIQ- Revised second version (MIQ-RS)** [Butler et al. 2012]. It is a 14-item questionnaire that rates one's ability to imagine movements will be tested by a questionnaire. The questionnaire consists of 7

visual and 7 kinesthetic items and requires 25–30 minutes to administer. The tasks performed and imagined include functional and gross movements and are all performed from a sitting position. After imagining the movements, the participants use a seven-point Likert scale to rate the ease or difficulty of seeing and feeling the movements. A score of 1 represents “very hard to see/feel,” and a score of 7 represents “very easy to see/feel.” The MIQ-RS will be administrate by a expert examiner.

- **Quantitative EEG** will be performed at the conclusion of the clinical assessment. It will be recorded using an eyes open/closed paradigm for about 30 minute (including preparation time). Electrode placement will be in accordance with the international 10-20 system, using a standard electrocap (about 35 minute administration time). The activity in 21 derivations will be recorded over the whole scalp (with Electro-Encephalo-Gram amplifier).

Pain and sensorial dysfunction assessment (for both treatment and control groups)

Pain assessment and sensorial dysfunction after stroke will be done accordingly the guidelines of the European Pain Federation (EFIC) with patient-reported questionnaires, including:

- Visual Analog Scale (VAS), it is a 10 cm scale with mm intervals giving a range from 1 to 100 mm scale (McCormack et al, 1988; <1 minute to complete)

- Short form of McGill pain questionnaire (SF-MPQ) consists of 15 descriptors (11 sensory; 4 affective) which are rated on an intensity scale as 0 = none, 1 = mild, 2 = moderate or 3 = severe. Three pain scores are derived from the sum of the intensity rank values of the words chosen for sensory, affective and total descriptors. (Melzack et al. 1987; about 10 min. administration time)

- Neuropathic Pain Symptom Inventory (NPSI) consists of 12 descriptors (subdivided in burning posontaneous pain, pressing spontanerous pain, paroxysmal pain, evoked pain and paresthesia/dysesthesia) which are rated on an intensity scale from 1-10, a total intensity score is give on a 0-100% scale (Bouhassira et al. 2004; about 2 minutes administration time).This test will be also used to stratify patients accordingly to their phycometric properties.

- Sensory tests of mechanical allodynia, touch and temperature sensations tests accordingly to international guidelines (Cruccu et al. 2010).

- Dolorimeter measure on trigger points

Biomarkers testing (for both treatment and control groups)

Blood withdrawal and preparation will be done at all time points with the following schedule: 10cc of serum for protein factors biomarkers will be prepared at T0, T1 and T3 and 5cc of total blood for genetic biomarkers will be prepared only at T0. The biomarkers to be tested by biologists are:

- **at T0 and T3**
  - **Metabolic/damage markers to be tested by hospitals (routine tests):** Creatin kinase, LDH, troponin, Eritropoietin, Emoglobin, C-reactive protein
  - **Vascular/BBB Marker to be tested by Partner 6 (University of Trieste):** MMP – 9
  - **Brain markers to be assessed by Partner 6:** NT – 3 and S100β
  - **Vascular/BBB markers to be assessed by Partner 6:** VEGF, VCAM
  - **Brain/Miokine markers to be assessed by Partner 6:** IGF 1/IGFBP3
  - **Immune system markers to be assessed by Partner 6:** IL-6, Fractalkine

- **at T1 and T3**
  Muscle markers to be tested by Partner 6 (University of Trieste): Irisine, Myostatine, Follistatine, C-terminal Agrin Fragment (CAF22)

Brain markers to be assessed by Partner 6: BDNF, Glial fibrillary acidic protein (GFAP)

Pain markers to be assessed by Partner 6 and Partner 7 (University of Nova Gorica): Serotonin, Substance-P

Genetic markers for therapy responsiveness to be assessed by Partner 6: BDNF-Val66Met polymorphism

Genetic markers for pharmacogenomics to be assessed by Partner 6 and Partner 7: ABCB, CYP, DPYD, GST, NAT, SLC, TPMT, SLC, VKORC1, SULT1A1, UGT, OPR, COMT, HRH, ADRA2A, MC1R, DRD, HTR CNR1, GABARP, GRIA, GRIN, GRIK, TRP, P2XR, SCN, CACNA.

Description of the motor rehabilitation in the control group

At T0 - The **standard motor rehabilitation** program for the stroke patients, starts in the neurology ward 24-72h (T0) after their recovery, because it is important that the patient is as active as possible and as soon as possible after stroke. The assessment done at T0 intends to identify any movement problems, also taking into account any health problems that the patient could had before the stroke, so that care can be organized consequently.

Between T0 and T1 – **Motor rehabilitation** during the early phase is composed by a **daily training session of about 75 minutes, for 5 days a week**. The therapy also aims at preventing joint stiffness or muscle tightness. Very often the patients in the first days of recovery after stroke are confined to bed because of clinical instability. In this case, the therapy consists in **changing the** position to improve the posture and balance, and make the patient more comfortable. In the case of one-sided paralysis, correct positioning and early movements are important to prevent spasm or injury. Accordingly, the physiotherapist starts with movements of the arm and leg paralyzed, searching for the attention of the patient about the part of the body moving. Subsequently, the patient is moved in the sitting position with legs out of the bed, to start the training balance program. When clinical conditions are stabilized, the physiotherapist starts the transferring training between bed and wheelchair. The transferring training is improved through the increase of the repetitive exercises; moreover the physiotherapist continues with movement exercises for the arm and leg paralyzed, increasing the repetition number.

Between T1 and T3 (min duration 4 weeks – in this case T3=T2 -, max duration 12 weeks) - The motor rehabilitation program consists in exercises from the Bobath's Method (neuromotory rehabilitation) and Perfetti Method (neurocognitive rehabilitation based on the perceptive experience of the body in order to recover the functional body's movements). These exercises will last 1h and will be done 5 days/week at incremental steps until the end of the rehabilitation.

STEP 1-After reaching a good control of the trunk, patients will start the motor training to reach and maintain the standing position. The training includes balance-specific activities, and more general strengthening activities, and progression to more challenging training activities. Rehabilitation of balance is made with exercises with closed eyes in standing position, with scales for calculate the weight on each leg.

STEP 2-After, stable maintenance of upright postion, patients start the walking training using an appropriate aid, with specific exercises for the walk, at the beginning in the gym and when the patient has obtained stability and safety, the training starts in the hallway of the department and in a second time also outside also on dismal grounds to improve balance and stability to reach the highest autonomy at the discharge.

STEP 3- After achieving a good balance and a good resistance to effort, patients start the stairway training, using the handrail or the aid.

Cognitive rehabilitation in the control group:

Cognitive rehabilitation in the control group is carried out only in the form of occupational therapy and speech therapy, when needed.

Description of the combined cognitive and motor rehabilitation in the experimental group

Experimental cognitive rehabilitation training

The cognitive rehabilitation training will be performed from T1 to T3.

The duration of each cognitive session will vary between 30 and 50 minutes. At the beginning, the training will last less time (20 minutes in order to complete at least 1 exercise) and duration will increase in the following sessions (up to 50 minutes in order to complete at least 5 exercise in each session). Three cognitive sessions will be performed every week for a minimum of 4 weeks up to a maximum of 8 weeks.

We prefigured the presence of 10 different exercises, training 5 different sub-components of executive functions (working memory, planning, inhibition, shifting and attention). Every exercise can be performed directly tapping on the screen and both reaction times and accuracy can be evaluated. All exercises are designed with increasing difficulty levels and the patient goes to the next level when he correctly answers to at least 75% of the stimuli of the current level. As a general method of intervention the Goal Management Training (GMT) will be offered to improve the ability to plan activities and to structure intentions (Levine et al., 2000).

Unlike common treatment paradigms, where only impaired sub-functions are predominantly treated, patients in the MEMORI-Net rehabilitation program will perform the complete set of exercises, in order to promote the activity of fronto-parietal circuits and the increasing recovery of Central Executive Network. The expectation for this procedure is that it should enhance the transversal power of the treatment, and eventually produce wider generalization to real life.

1) Working Memory

• **Updating:** during working memory updating tasks, a continuous retrieval of information is required, in order to maintain accurate representations of information changing over time. Typically, arrays of stimuli of various lengths (e.g. list of letter, digits, shapes or pictures) are presented sequentially. Participants are requested to evaluate a selected stimulus as to whether it matches another stimulus presented n-time before (from 1- up to 3-items back). Task difficulty is gradually enhanced by manipulating memory load. For example, in a verbal task, participants are asked to monitor a series of letters and to respond whenever they see a letter that is the same as the one presented either 1, 2 or 3 trials previously.

• **Manipulation:** during working memory manipulation tasks transformation and substitution of presented information is required, in order to replace the memory content with the newest information. Typically, arrays of stimuli (e.g. list of letter, digits, shapes, pictures or spatial locations) are presented sequentially and must be remembered in a given order. For example, participants have to reorder the sequence into alphabetical order across a fixed delay period, or to change the selected letter by alphabet arithmetic (e.g. reporting the letter subsequent to the one presented).

2) Planning

• **Everyday Planning:** naturalistic tasks are employed, such as planning a grocery-shopping trip in a shopping mall, using a map (either of a real existing place or not), or planning a route in order to visit a number of places on a city map. The goal of these tasks is planning and choosing the correct route from a starting point to a specific end point, visiting a given number of locations, following some rules and instructions. The number of places to visit and instructions is gradually increased to improve problem-solving skills, taking into account structured environmental feedback.

• **Action Sequences:** the exercise consist in putting in right order a series of words that describes, for instance, the various steps of a recipe to cook a dish or another complex action.

3) Inhibition

• **Go-No-Go Task:** it is used to train patient's capacity for sustained attention and response control. For example, a go/no-go task requires participants to perform an action, given certain stimuli (e.g., press a button – Go condition) and inhibit that action under a different set of stimuli (e.g., not press that same button - No-Go condition). The task can be performed in different sensory modalities (either visually or acoustically: for example pressing a button with the right hand each time a particular cue or sound is presented and not pressing in response to other cues or sounds).

• **Modified Stroop Tasks:** This set of exercises are variants of the original Stroop task created by replacing the word-colour conflict with different conflicting type of information (e.g. number-magnitude conflict). These tasks aim to train patients to control an irrelevant automatic response and provide a relevant secondary response. For example, in the Numerical Stroop Task, digits are presented visually and they can be physically large or small, irrespective of their actual values. Patients can perform a physical or a numerical size judgement task. In the numerical task, participants respond to the values and ignore the physical sizes and in the physical task, participants respond to the sizes and ignore the values. The training can be performed also with a words/pictures set of stimuli and with increasing difficulty.

4) Shifting

• **Task switching:** these exercises train the ability to switch tasks rapidly in order to respond flexibly in a changing environment. Different switching behaviours can be encouraged depending on whether the switch is between sets of rules (rule switch) or sets of physical features (perceptual switch). One exercise requires patients to identify the colour or the shape of stimuli in matrices, and then apply that rule for the subsequent trials. In addition, participants are required to implement the appropriate set of response rules, which can be arbitrarily modified from the therapist during the training (e.g., key press for red or circle until a cue is presented, then key press for blue or triangle). Task switching requires participants to move visuo-spatial attention away from one set of features in order to selectively attend to the alternative feature set.

5) Attention

• **Barrage:** A set of tasks requiring cancellation of stimuli (letters, pictures, digits, geometric shapes) is proposed for the training of patients' spatial and selective attention. The request is to mark the target stimulus/i among distractors. It is possible to modify the degree of difficulty by increasing either the number, the size or the perceptual properties of the elements (targets and distractors).

• **Oddball Task:** this is a target detection task where patients respond to target stimuli that occur infrequently and irregularly within a series of standard stimuli. In a visual oddball task, there might be a 95% chance for a square to be presented and a 5% chance for a circle. When the targets (e.g. circles) appear, the subject must make a response, such as pressing a button or updating a mental count. The same paradigm can be performed in different sensory modalities (e.g., a different tone among auditory stimuli) or inserted in a virtual ecologic context.

• **Dual Task:** dual task paradigms require working memory and attentional control. This set of exercises is based on the combination of two tasks to be performed simultaneously. Single tasks from the Barrage set of exercises are selected and coupled with an auditory task (e.g. listening and responding to a tone or a rhythm) so that they do not compete for the same processing mechanism.

Experimental motor rehabilitation training

The motor rehabilitation training will be performed from T1 to T3.

Patients enrolled in the neurofeedback (NFB) rehabilitation program at T1 are expected to show a motor deficit such as raising the hand, hemiplegia, paresis, difficulty of walking, deficit with stable posture (normal stance position). Duration of each neurofeedback session will vary between 50 and 60 min which comprises the whole session with EEG electrodes preparation and the breaks. However, at the beginning, also in consideration of the patients' conditions, the training will last 10 min of imagery plus 20 min for mounting the cup and calibration and in the following sessions up to 30 min of imagery plus 20 min for mounting the

cup and calibration. Three NFB sessions will be performed every week for up to a maximum of 8 weeks from the onset of NFB treatment and in total, participants will perform a maximum of 24 training sessions.

The NFB protocol will include multiple feedback site (one or two of the most activated electrode). For this, from 5 to11 EEG electrodes will be placed over sensory-motor cortex according to the International 10/20 system [Jasper, 1958]. The NFB treatment will consist of 2 phases: a calibration and visual feedback training.

The calibration phase will be performed to adjust the EEG classifier parameters [Ritter et al. 2009]. An image of the hand, ("imagine paretic hand opening and closing") or no image ("rest") will be displayed for 5 s over a cross-shaped icon at the center of a monitor, to instruct participants to perform the task. This training run will consist of 40 trials, with 20 trials per run, presented in randomized order. This procedure will be repeated twice, for a total of 80 trials. Each run will last about 400 seconds, for a total time of the initial session of about 12 minutes, including inter-run pauses.

SMR represent the patient's motor intention during the task [Ritter et al. 2009; Pfurtscheller et al. 2009]. The parameters in SMR will be determined by the EEG obtained during the calibration session. The power spectrum of alpha band (8–12 Hz) and beta band (12–30 Hz) components of SMR in the bilateral hemispheres will be used to judge whether the patient is in "imagined paretic hand moving" or "rest" state. To highlight the most reactive SMR, the greatest active electrode will be determined by analysis and inspection of the elaborated data. The selected electrode will then remain the same during the following trials.

The feedback phase, allows patients with different deficits to interact with personalized visual Videofeedback. For example, patients with a deficit of the pretension could be presented with a film in which the hand of a subject, resting on a flat surface, moves to have a fine grip on an object (i.e. grasping a coffee cup) placed on the same surface, lifting it, putting it on the floor and returning to the starting position. Patients with spasticity of the hand could be presented with a film in which a subject opens and closes his hand. The velocity of videos will reflect the accumulated output value of SMR classification. Thus, the video will not move if a decrease of SMR will not be clearly observed. Each training run will consist of 8 trials, with a duration of about 4 (bad performance) to 11 (good or intercropped performance) seconds. Inter trial duration will last 3 seconds, so the duration of one run will amount from 65 to 110 sec. Ten training runs will be recorded per session, for a total of 80 trials. Inter run pause will last 80 sec; the total duration of this phase will then be approximately form 22 to 30 minutes.

EXPECTED OUTCOMES

The observed changes in the study population will be considered beneficial if at T3 the treatment could lead to an improvement from baseline in one among the following efficacy areas at the indicated endpoints:

- **Primary efficacy outcomes:**

Improvement of cognitive deficits, measured with the following endpoints:

• Patients scoring at the Montreal Cognitive Assessment (MOCA) should reach at least 26/30 (normal range);
• The Frontal Assessment Battery (FAB) score should improve up to 13/18 or more;
• Patients will also complete the BRIEF-A questionnaire (Behavior Rating Inventory of Executive Function - Adult Version: https://www.hogrefe.it/i-nostri-progetti/lavori-corso/brief-a-behavior-rating-inventory-of-executive-function-adult-version/) to judge their executive functioning on every day life, as a measure of generalization of the training. Moreover, the Global Executive Composite (GEC) can be used as a summary score that incorporates all of the BRIEF-A clinical scales (higher scores indicate that the patient encounters greater difficulties e.g., 65 or greater is considered clinically significant for executive function impairment).

Improvement of motor deficits, measured with the Fugl-Meyer Assessment (FMA). The FMA is a clinical scale focused of body function damage. It assess a motor impairment severity on 5 domains (motor, sensory, balance, range of motion, joint pain). The motor functions examined in FMA take into account movement components at subsequent measurement points, rated pre- and post-BCI training. The means and standard deviations of the FMA scores at the subsequent measurement points and means and standard deviations of the differences between those measurements pre- and post-BCI training, will be calculated. Greater motor severity is indicated by lower motor scores and is correlated with lower functional ability, such as spontaneous arm use for feeding, dressing and grooming, or walking at functional gait speeds. Positive effects of neurofeedback treatment will be considered if, after the training, higher motor scores in the FMA will be observed. An average improvement of about 3 in the FMA scores is expected (Hallet, et al., 2001; Richards et al., 2008; Tung et al., 2013). The neurofeedback treatment will also be considered efficacious if an improvement of at least 1 point will be measured in any subscales, or global scales measured by the BARTHEL INDEX, NIH-NINDS, TCT or RANKIN MODIFIED SCALE.

- Secondary efficacy outcome:

1. Reduction in pain sensation, treatment will be considered efficacious if an improvement in at least one of the following pain questionnaire scales VAS; McGill, NPSI will give a reduced score by at least 30%.

Other parameters

Reduction in event-related desynchronization. Since brain activity can be self-regulated by NFB, then changes of EEG activity after the training are expected. Thus, the amplitude of SMR changes will be estimated as a feature of cortical excitability of the sensorimotor area. Reduction of SMR is termed event-related desynchronization (ERD), and is expressed as the percentage power decrease in relation to the 2-s reference interval before the task. ERD at the alpha and beta band will be determined. ERD pre- and post-NFB training will be compared. Negative ERD values mean decrease of the power spectrum during motor imagery, indicating cortical activation. Since ERD values indicate decreases of the power spectrum during motor imagery, indicating cortical activation, greater ERD reduction is expected. We will estimate the amplitude change of SMR as a feature representing cortical excitability of the sensorimotor areas. Based on Shindo and colleagues (2011), beneficial effects will be considered if the mean reduction of ERD values will be around 30%.

Positive correlation of FMA scores with ERD changes. A positive effect of NFB on motor deficits will be considered if improvements of FMA scores will positively correlate with ERD changes.The positive correlation between the change in FMA scores and the value of ERD will indicate that improvement in motor recovery is associated with increasing activation during the NFB therapy.

Improvement of ability to image of movements. The MIQ-RS is a 14-item questionnaire that rates one's ability to imagine and consists of 7 visual and kinesthetic items. After imagining the movements, the participants use a seven-point Likert scale to rate the ease or difficulty of seeing and feeling the movements. A score of 1 represents “very hard to see/feel,” and a score of 7 represents “very easy to see/feel.” Benefit effects from neurofeedback treatment will be considered if higher rate of visual and kinesthetic items will be observed after the treatment. Moreover, a positive correlation between scores on the FMA and performance on the MIQ-RS will be expected. Efficacy NFB will be considered if the total MIQ-RS score and its kinesthetic and visual subscores will be significantly higher in the experimental group than in the control group, with a mean total score higher of about 5 points for the visual motor image and of 3 points for kinesthetic motor image. A positive correlation between scores on the FMA and performance on the MIQ-RS should be expected.

Changes in serum levels of brain, muscle, inflammation and pain biomarkers. Expected changes if treatment will be efficacious for: pain at least 1 biomarker decreased by 10%; brain at least 1 biomarker increased by 10%; muscle at least 1 biomarker increased by 10%, immune function at least 1 biomarker decreased by 10%

METHODS a – RANDOMIZATION:

Each subject will receive a **Clinical Study Participation Card** and a numeric number generated by the ICT platform created for the management of this study, which will also serve for assigning the patients randomly to the control or experimental group. This ID card will have the details of the study on it and an emergency contact number. The subject should carry this with him/her all times during the study in case of emergency to allow other healthcare professionals to obtain information about the trial medication and procedures. This number should only be used in case of urgent medical concern. All other inquiries will be answered directly at the Study site. Written informed consent should be acquired from the patient before starting any study procedures required by the protocol. Prior to obtaining informed consent the details of the study must be discussed with the patient. The **Informed Consent Form (ICF)** must be signed and dated by the patient and by the Investigator or his/her designee. A copy of the ICF should be given to the patient the original should be retained with the source documents.

Anonymization. The IT platform will use the codes to randomly assign patients to the control or experimental group and anonymize the data that will be sent to the data analysis center. The random assignment of patients to one of the two arms of the study will take place at the Stroke Unit facilities of the 3 neurology clinics involved.

The study will be conducted following present regulations and in accordance with the ethical principles of the Declaration of Helsinki. The clinical protocol will be approved from the Institutional Review Board (IRB), before starting the study. The enrolment in the study will be completely voluntary and no financial incentives will be provided.

METHODS b- DATA COLLECTION:

Personal patients data collected during dispensing the treatment by Neurology Clinics (**Coordinating Center and Partners 1, 2**) and Rehabilitation Centers (**Partners 3, 4, 5**) will be retained in a safe, secure location and stored under the appropriate informatics safety rules.

At each center dispensing the treatments, the PIs are responsible for the evaluation of subjects compliance throughout the trial. The PIs or delegated site staff will dispense the experimental (Protocol A) or the standard control (Protocol B) therapy and will maintain an adequate record of the receipt and distribution of all intervention-related records. The site personnel are responsible for intervention accountability that will be performed prior to subject treatment. Patients and medical staff will be not blinded to the treatments.

Privacy rules in sample collection. Human samples will be kept anonymous by using numerical coding references, will be used only within the aims of the present proposal and destroyed at the end of the trial.

Letter to the general practitioner. At the recruitment in the study, a letter will be sent by the PI to the general practitioner of each patient to provide information on the clinical trial, possible consequences and benefits.

A **Case Report Form (CRF)** is a printed, optical or electronic document designed to record all of the trial-required information on each trial subject. The CRFs will be completed within 7 days of the subject's visit, completed for all subjects who have signed the informed consent, including screen failures and medical history (or related documentation) to support verification of all inclusion and exclusion criteria.

Withdrawal from study. Patients may withdraw from the study at any stage of the treatment by filling and signing the withdrawal form and submitting it to the PI. As a consequence of the withdrawal, patients will be submitted to the standard rehabilitation protocol in use at the center where they are referred to.

If a subject is **discontinued prior to the completion** of planned visits, the investigator will make every effort to perform the Early Termination Visit, Taper period, and Safety Follow-up telephone contact. Discounting of a subject will follow the subsequent rules:

- an early or late visit is not a reason for subject discontinuation.

- a subject may voluntarily discontinue at any time.

- the PIs may, at their discretion, discontinue the subject at any time.

- a subject may be discontinued if he/she requires a change in therapy requiring excluded concomitant medication.

- a subject may be discontinued from the trial if subject symptoms worsen during the course of the trial.

Incidental Findings might arise because of identification of unknown pathogenic microbes. If, such case should occur, a contact person will be contacted for critical evaluation of the clinical relevance of the unexpected finding and deciding about disclosure. The research team will not influence the decision.

Documentation

At each visit, the subject will be questioned regarding **new adverse events** or changes to ongoing adverse events. Details regarding all adverse events will be documented, including symptoms, onset date, severity, actions taken, relationship to the medical treatment, outcome of the event and, if resolved, date of resolution.

At each visit the subject will be questioned regarding their **new concomitant medications** or changes to ongoing medications. Details regarding the concomitant medication will be documented, including medication name, indication for use, dose, start date, stop date or, if ongoing. If no changes in medication have occurred, documentation will report it.

Subject questionnaires are considered source data and will be reviewed by site staff after the subject has completed their responses to ensure completeness and accuracy.

METHODS c - SAMPLE SIZE AND STATISTICAL ANALYSIS:

The size of the sample (60 patients per treatment and control groups) has been estimated taking into account the typical outcomes of current rehabilitation therapies. The needed sample size was calculated on the basis of statistical power analysis, and the expected changes in the major scales used in cognitive and motor rehabilitation. Details of the different analyses performed for each items is reported below. All provisional estimation of sample size were performed considering the t-test (two tailw) as primary statistical tool to evidence significant effects of the treatment, unless the biomarkers for which a paired t-test is necessary because patients may have different baseline levels and therefore changes should be quantified individually.

The primary end-point “Improvement of cognitive deficits”, focused on deficits measured by MOCA and FAB assesments, will consider for MOCA precondition score, the range 15-20, mean 17.5 and an average endpoint of 26 for the Experimental group and 22 (=mild cognitive impairment) for the Control group (Range of test: 0 to 30) and for FAB precondition score, the range 8-12 and an end point of 13 or more (Range of test: 0-18). Considering for the MOCA test a Type I error alpha= 0.05 and a Type II error beta = 0.05 (statistical power of at least 95%) or beta = 0.10 (statistical power of at least 90%), the needed sample size leading to a statistically significant difference between the two groups after treatment was **calculated in 58 (Power 95%) or 46 (Power 90%) patients for each group**. We calculated this number using the software Gpower (www.gpower.hhu.de/) and assuming the measured MOCA for patients in the acute post-stroke

phase as having mean = 17.5, SD=5; vs. MOCA target values after therapy, mean = 22 for the Control group and mean = 26 (normal value) for the Experimental group, with an SD = 6.5 in both cases, the calculated effect size was d= 0.615 and the actual Power = 0.9505.

We will perform both univariate and multivariate analysis. As univariate analysis, we will performed partial correlations using the log-transformed biomarkers data, and the values of the different cognitive and motor test scales, controlling for gender, BMI (or other possible demographic characteristic), in order to detect association between each predictors and the dimensions of the effect of treatment.

Multivariate analysis will be also performed to explore association between predictors. More specifically, the partial least square regression procedure (PLS) was identified as the best suited method for this purpose because it is particularly useful when predictor variables are highly correlated or the number of predictors is considerable in respect to the number of cases. This method extracts, at first, several latent factors that explain as much of the covariance as possible between dependent and independent variables and then computes a series of score (Variable Importance to the Projection or VIP) that help to identify predictors that best contribute to the association with the dependent variable. As with partial correlation analysis, also for PLS we will use each scale of the cognitive and motor assessment tests as dependent variable and biomarkers and the interaction effect between gender and BMI as independent variables.

METHODS c – ORGANIZATION AND FEASIBILITY

Participating Centers: The 9 centers that will participate to the study are:

- Coordinating Centre – **Neurology Clinics**, ASUITS - Trieste, Italy.
- Partner 1 - **Neurology Clinics**, Ospedale San Tommaso dei Battuti, Azienda ULSS n.4 "Veneto Orientale" - Portogruaro, Italia
- Partner 2 - **Neurology Clinics**, Splošna Bolnišnica Izola - Izola, Slovenia
- Partner 3 - **Medicina Riabilitativa** – ASUITS Ospedale Maggiore - Trieste, Italy
- Partner 4 - **Rehabilitation Center**, Bolnišnica Sežana - Sežana, Slovenia
- Partner 5 - **Medicina Fisica Riabilitativa**, Azienda ULSS n.4 "Veneto Orientale" – Portogruaro, Italia
- Partner 6 - **University of Trieste** - Trieste, Italy
- Partner 7 - **University of Nova Gorica** - Nova Gorica, Slovenia
- Partner 8 - **Science and Research Centre Koper** – Koper, Slovenija

Organization: Stroke patients will be recruited from Italian and Slovenian hospitals which participate to MEMORI-net a recently established international network including experts in clinical research on stroke, rehabilitation, pain and neuronal plasticity, sponsored by the Italy/Slovenia Interreg V Programme (www.ita-slo.eu). These centers are tertiary care Hospitals, and collectively approximatively 200 ischemic stroke patients per year undergo rehabilitation treatment.

Patients will be recruited at the the Neurology Clinics of two Italian and one Slovenian hospitals, namely the Coordinating Center and Partners 1 and 2. The partner centers 3, 4 and 5 have a wide experience in caring for patients with stroke, and can provide a multidisciplinary approach for rehabilitation. Partner 6, will be responsible for laboratory measurements of biomarkers of neuroplasticity and muscle physiology. Moreover, the Partner 6 will perform all the statistical analysis of data collected at the different clinical centers. Partner 7 is an expert in molecular underpinnings of pain and will measure genetic biomarkers of pain sensitivity. Finally, Partner 8 has a specific expertise in motor deficits measuring and recovery through virtual reality, and will provide support to each rehabilitation center on this matter.

Feasibility: One of the key points of this study is the possibility to collect clinical data on efficacy and safety of rehabilitation with minimal impact on the patients and involvement their families. For this purpose, we will collect data using computer-based analysis recorded at home and then sent to Partner 6 via an ICT platform

generated for this study. We will also conduct the study reducing the number of visit and using phone calls/email contacts to keep feed-back with the patients and their families. This strategy will reduce not only the costs of the protocol, but also the burden of the patients and their families participating to the study, thus facilitating enrolment and compliance to the research.

METHODS e –PRIVACY:

The study will be conducted following present regulations and in accordance with the ethical principles of the Declaration of Helsinki. The clinical protocol will be submitted in Italian language to the Institutional Review Board (IRB), before starting the study. The enrolment in the study will be completely voluntary and no financial incentives will be provided.

Staff members will follow the **Good Clinical Practice** (**GCP**) guidelines provided by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and local regulations when obtaining informed consent. Each subject will receive a personal copy of the signed consent.

For ensuring **protection of Human Subjects** the following will apply:

- No investigator may involve a subject in research unless an Ethic Committee approved, legally effective informed consent of the subject has been obtained.

- Consent will be sought only under circumstances that provide the prospective subjects sufficient opportunity to consider whether or not to participate.

- Information given to the subject/parent/legal representative will be written/explained in a language understandable to the subject/parent/legal representative.

- No informed consent may include exculpatory language through which subject/parent/legal representative waves her legal rights or which releases the investigators, Funding body, or Institution from liability for negligence.

Here are the main points we will ensure to data protection and subject privacy:

Protection of Personal Data: we will comply with the Directive 95/46/EC of the European Parliament and transposed National internal laws (For Italy: New General Data Protection Regulation- UE 2016/679, in effect on May 25th 2018).

- Management of human samples will be managed accordingly the privacy roles, by using an numerical coding system and samples will be destroyed at the end of the trial.

- **ID CODE**: Subjects will be assigned a randomize ID code, both for ensuring anonymity and for trial randomization by number at the beginning of the study. Then, patients and medical staff will not be blinded to the treatments.

- In line with the **Singapore statement** on Research Integrity (2010), data will be stored and backed up in protected servers during the lifecycle of the project and for at least 5 years after completion.

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